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LIFE HISTORY AND DEVELOPMENT OF SOME FUNGI



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THE PERFECT STAGE OF ACTINONEMA ROSAE¹

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(WITH PLATE XIII)

Perhaps no plant disease has been more widely observed or is more generally known, both in Europe and the United States, than the black spot of roses caused by the parasitic fungus *Actinonema rosae* (Lib.) Fries. The spots, which are more or less circular in outline, are characterized by a very irregular, fibrillose border. This fibrillose character is due to the radiating strands of mycelium which occur beneath the cuticle. Appearing among the mycelial strands are numerous dark specks, the fruit bodies of the fungus. The spots may be isolated and confluent, or so numerous as to involve the entire upper surface of the leaf. Plants which are attacked become defoliated early in the season, and the leaf buds, which should remain dormant till the next year, often open late in the season. As a result, the plant is weakened so that it blossoms poorly or not at all in the following season.

Since very little is known concerning the life history of the fungus and the development of the *Actinonema* stage, an attempt has been made by cultures on artificial media and on the host to furnish a more satisfactory knowledge of this interesting organism. Before giving an account of this study it may be well to state briefly the characters of the vegetative and fruiting structures of the rose *Actinonema*.

The vegetative body of the fungus consists of two parts, the subcuticular mycelium and the internal mycelium. The subcuticular mycelium is immediately underneath the cuticle, being above the outer wall of the epidermal cells. It consists of branched, radiating strands of mycelium which anastomose, making a network. Each strand consists of several filaments united together, either side by side or sometimes superimposed. At the right of the acervulus in fig. 1 is shown a cross-section of one of these strands. The internal mycelium penetrates the mesophyll of the

¹ Contribution from the Department of Botany, Cornell University. No. 143.

leaf and furnishes nutriment for the subcuticular part. It is connected with the latter by occasional hyphae which penetrate the epidermal cells or pass between them.

A section of the fruit bodies or acervuli perpendicular to the surface of the leaf shows that they are formed between the cuticle and the outer wall of the epidermal cells. They are consequently flattened. The stroma of the acervulus is seated directly on the epidermal cells and consists of a very thin layer of small, hyaline to yellowish, pseudoparenchymatous cells. It is connected with the internal mycelium below by hyphae which extend either through or between the epidermal cells into the mesophyll. Laterally the stroma is connected with the subcuticular mycelium. There is no wall or membrane of fungous tissue covering the acervulus. On the upper side of this stroma certain cells are formed which bear the conidia. These conidiophores are not prominently differentiated in form from the other stromatic cells, but are slightly elongated upward. The conidia are hyaline, 2-celled, and oval to elliptical in outline. They are usually somewhat constricted at the septum. The conidia are formed on the somewhat pointed upper ends of the conidiophore layer. The great numbers which are produced cause such a pressure that the cuticle is finally ruptured. The cuticle, which is the only covering for the acervulus, is thus thrown back irregularly, exposing the mass of conidia and permitting their escape.

While the spots together with the mycelial strands and acervuli appear dark, this color is not due to the fungus, which is almost colorless, but to the disintegration of the cells below the spot.

Development of acervuli

It is from the subcuticular mycelium that the acervuli arise. At certain definite points the mycelium begins to form a stroma, which increases in a centrifugal manner, forming a more or less circular stromatic layer. Certain cells of this stroma which are to give rise to the conidia are directed upward as short stalks. These increase in size, forming a closely aggregated layer standing perpendicular to the stroma. Meanwhile, the mesophyll tissue directly below the acervulus is being disintegrated and a dense

tangle of fungous filaments is formed in its place. From the perpendicular cells arising from the stroma a cell is cut off by a transverse septum. This cell enlarges into an oval body, the conidium, which soon becomes septate. As the conidia are increasing in size, the pressure on the cuticle above becomes greater and greater, so that it is at length broken, leaving the margin of the exposed acervulus irregularly torn (fig. 1). Sometimes a central papilla is present which marks the place where the cuticle will rupture. At maturity the conidia are oval to elliptical and 2-celled. They are hyaline and $18-25 \times 5-6 \mu$. They may be unequally septate, either straight or subfalcate, and often so deeply constricted at the line of septation that the halves fall apart readily. Several large granules and guttulae are normally present (fig. 3).

Germination of conidia

The conidia germinate within 24 hours in bean agar or in hanging drops of water. Each of the cells may first enlarge, becoming more or less spherical and vacuolate before the formation of the germ tube. Frequently only one of the cells germinates by the formation of one or two germ tubes (fig. 3). No formation of colonies was secured in poured plates of bean agar, although the fungus grows slowly when the conidia are planted on the surface, forming a small, prostrate, tawny colony. Apparently growth ceases as soon as the reserve food material within the conidium has been utilized in the development of the short hypha. This seems to occur when the hypha is 10-20 times the length of the conidium and may have become branched with several septa. If such conidia are cut out with as little of the surrounding medium as possible and transferred to bean pods, using ordinary sanitary precautions, and if the medium is spread out so as to bring the germinating conidium in contact with the pod, further growth may be induced. In two or three weeks small colonies are formed. At first the mycelium is whitish, changing to a pinkish color and becoming pale brown to blackish with age. The colonies do not spread out on bean pods, but form knots of fungous tissue often one-half as high as the diameter of the colony. The tissue of the bean which is attacked becomes blackened in a fibrillose manner,

simulating the blotch on the leaves. Conidia are formed readily on the ends of the hyphae. Such conidia are often so strongly constricted at the septum that each cell is round. There is, then, little surface of contact between the cells and they are readily separable one from the other. These spherical halves germinate in the normal manner (fig. 2). Acervuli apparently like those on the leaves are also formed on bean pods in the blackened areas. These bear conidia like the typical ones from rose leaves.

Systematic position of the conidial stage

The genus *Actinonema* is usually placed by systematists in the Sphaeroidaceae.² This family is a group of imperfect fungi possessing a pycnidium of the type present in *Ascochyta*, *Sphaeropsis*, etc. The pycnidium or conceptacle is more or less oval in form, with a membranaceous wall of fungus tissue, usually opening at the apex by a minute pore. Some writers speak of the fruit bodies of *Actinonema* as pycnidia³ or perithecia.⁴ FRANK⁵ considered them as very flat spermagonia ("des sehr flachen Spermagoniums"). SORAUER⁶ speaks of them as small astomate pycnidia ("die kleinen mündungslosen Pykniden").

It is very evident from the foregoing account that the conidial stage of the rose *Actinonema* is not of the type in which the conidia are borne in a pycnidium or perithecium. The conidia are borne in an acervulus resembling that found in the Melanconiales, as exemplified by *Gloeosporium*, *Marsonia*, etc. SCRIBNER⁷ has correctly figured the structure of the acervulus and says that while the fungus from analogy is placed with the sphaeriaceous fungi, no perithecia-like or pycnidial structures have been observed.

Partly because of the different interpretations of the morphology

² SACCARDO, P. A., Syll. Fung. 3:408. 1884; also LINDAU, G., Sphaeropsidales in ENGLER and PRANTL'S Pflanzfam. 1:369. 1900.

³ LINDAU, G., loc. cit.

⁴ MASSEE, G., Diseases of cultivated plants. 428. 1910.

⁵ FRANK, A. B., Die Krankheiten der Pflanzen. 621. 1880.

⁶ SORAUER, PAUL, Handbuch der Pflanzenkrankheiten. 406. 1908.

⁷ SCRIBNER, F. L., The black spot on rose leaves. Rept. U.S. Dept. Agric. 366-369. pls. 8, 9. (1887) 1888.

of this fungus it has been variously named by different workers. In 1849 the name *Actinonema rosae* (Lib.) Fr.⁸ was employed. In 1853 BONORDEN⁹ described it as *Dicoccum rosae*, one of the Hyphomycetes. He says that the fungus forms small, closely aggregated pustules of a brown green color which dehisce irregularly. From collections made in 1888-1889, BRIOSI and CAVARA distributed the species under the name *Marsonia rosae* (Bon.) Br. & Cav.¹⁰ because they recognized the acervulus type of fruit body which is characteristic of the Melanconiales. The 2-celled hyaline conidia suggested its position in the genus *Marsonia*.¹¹ I have been able to examine the specimens distributed by BRIOSI and CAVARA and have found them to be the same as the rose *Actinonema* in the United States. The drawing of acervulus and spores which accompany the specimens show the same structure.¹²

SACCARDO¹³ notes that *Marsonia rosae* (Bon.) Br. and Cav. resembles *Actinonema rosae* (Lib.) Fr. This same fungus was described by TRAIL¹⁴ in 1889 as occurring on roses in Scotland. He called it *Marsonia rosae*.

The characters of the genus *Actinonema* have changed from time to time since the genus was established by PERSEON.¹⁵ He applied the name to those forms on leaves and stems having radiate sterile mycelial strands. He describes two species, *A. crataegi* and *A. caulicola*, in neither of which perithecia or conidia were observed.

In 1828, FRIES¹⁶ included two species in the genus *Actinonema*,

⁸ FRIES, ELIAS, Summa veg. Scand. 424. 1849.

⁹ BONORDEN, H. F., Beiträge zur Mykologie. Bot. Zeit. 282. pl. 7. fig. 2. 1853.

¹⁰ BRIOSI and CAVARA, Funghi parassiti delle coltivate od utile. n. 97. 1889.

¹¹ La natura degli acervuli fruitifera, subcutanei ed erompenti, ci induce a riferire questo funghetto ai Melanconiei sezione delle Didymosporee Sacc. ove trova riscontro nel genere *Marsonia* pure a spore didime e jaline.

¹² Type material was received through the courtesy of the Bureau of Plant Industry, U.S. Dept. Agric. I am greatly indebted to Miss ETHEL C. FIELD of the same Bureau for some notes on the specimens. She finds that there is no apparent difference between *Marsonia rosae* of this collection and other European material which is labeled *Actinonema rosae*.

¹³ SACCARDO, P. A., Syll. Fung. 10:477. 1892.

¹⁴ TRAIL, J. W. H., Micromycetes of Inveraray. 46. 1889.

¹⁵ PERSEON, C. H., Mycologia Europaea 1:51-52. 1822.

¹⁶ FRIES, ELIAS, Elenchus Fungorum. 151. 1828.

A. padi and *A. crataegi*, the latter showing at length perithecia-like structures, but no conidia were observed. In 1829¹⁷ he employed the name *Actinonema* for the sterile state of fungi belonging to the Pyrenomycetes and Perisporiaceae. Later¹⁸ he characterized the genus as having a fibrillose, radiating mycelium, a delicate perithecium, and bilocular spores, and lists *A. rosae* as one of the species which often possesses only a sterile mycelium. SACCARDO¹⁹ employs these characters as given by FRIES and notes that the fruits have not been observed in many species. Of the 18 species of *Actinonema* which have been described, there are 8 species in which the spores were not observed at that time. The radiating fibrillose character of the mycelium has been used as the principal generic character for these species, thus employing the distinctive character as originally given by PERSOON. LINDAU²⁰ includes in *Actinonema* astomate pycnidial forms occurring on leaves. The pycnidia arise from radiately actinic strands of mycelium.

The genus *Marsonia* is characterized by having a *subepidermal* acervulus, in which are produced hyaline, 2-celled conidia, very similar to the conidia of *Actinonema*. Several species of *Marsonia* have been described, however, in which the acervulus is *subcutaneous*, as *Marsonia baptisiae* E. & E., *M. panatoniana* Berl., and *M. fructigena* (Rick.) Berl. BRIOSI and CAVARA recognized the true morphology of the rose *Actinonema* acervulus, but attached no significance to the fact that it was *subcuticular* and not *subepidermal*. The *Actinonema*-like character of the mycelium was not taken into account by them as indicative of generic position. TRAIL must have been of the same opinion when he named this same fungus *Marsonia rosae*.

Even though the subepidermal acervulus has been made one of the generic characters of *Marsonia*, it would seem that these subcuticular forms might properly be placed in this genus. On the other hand, we do not know the structure of the perithecia or pycnidia of other species of *Actinonema*. If we accept PERSOON'S

¹⁷ FRIES, ELIAS, *Systema Mycologicum* 3:266. 1829.

¹⁸ ———, *Summa veg. Scand.* 424. 1849.

¹⁹ SACCARDO, P. A., *Syll. Fung.* 3:408. 1884.

²⁰ LINDAU, G., ENGLER and PRANTL'S *Pflanzfam.* 1:399. 1899.

characterization of the genus, it has no fruit bodies, but consists only of sterile mycelium.

The rose fungus evidently, then, does not possess the characters of a typical *Marsonia*, nor does it agree with the original characterization of *Actinonema*. Whether these differences are worthy of good generic rank, separating it from both these genera, is a matter for consideration.

Development of the ascigerous stage

During the autumn of 1910, leaves attacked by the conidial stage were collected and placed in wire cages to winter out of doors. When some of these leaves were brought to the laboratory early in April and examined, shield-shaped structures suggestive of the perithecia of the Microthyriaceae were found to be present. At this time, however, no spores had been developed. Fig. 2 shows one of these perithecia as seen in surface view. Such preparations were made by stripping off the epidermis of the leaf together with the perithecia. By April 27 these perithecia had matured and were found to possess characters similar to the genus *Asterella*, a genus apparently including heterogeneous elements.

For the study of the development of the perithecia, material was killed in Merkel's fluid and stained with Flemming's triple stain. By killing material at different times during a period of three weeks, many of the developmental stages were obtained. Not all perithecia on the same leaf are in the same stage of development at the same time. Unfortunately the material was too far advanced for the study of fertilization and the immediate subsequent development. This in itself would be a very interesting study, since nothing is known of these phenomena in the Microthyriaceae.

The shield was found to be entirely separate in origin from the tissue which gives rise to the asci. It is formed immediately beneath the cuticle from the radiating strands of mycelium which now are thick-walled and dark brown in color. The strands themselves can be traced across the shield (fig. 4), showing that the growth begins at any point on the mycelial strand and new cells are added in a centrifugal manner. In this way a more or

less circular shield is formed, the elements of which are arranged in a radiating manner, especially noticeable at the margin of the shield. The cells which make up the shield possess thick, dark walls.

The shield varies in diameter from 100 to 250 μ , and may be more than one cell in thickness. In fig. 6 is shown a young stage in the development of a perithecium. The shield forms a thin layer above the epidermal cells or beneath the elevated cuticle. Beneath the epidermal cells, and above the palisade parenchyma, is an undifferentiated layer of fungous tissue, the stroma from which the asci later arise. This stroma is 3-6 cells in thickness and is made up of cells similar to those of the shield. Occasional filaments connect these two layers through the epidermal cells of the host. In fig. 7, when the fertile layer has increased and the fruit body has begun to be differentiated, the shield is still distinct and not connected with it at the margin. At this time the cells in the center of the young fertile stroma are thinner walled, with a more deeply staining content.

The asci are formed within the fertile stroma, arising from the basal portion, as shown in figs. 8 and 9. In this way the cells in the upper part of the fertile stroma persist, forming a delicate covering over the hymenium. The development of the asci within this fertile stroma is comparable with their origin in the apothecia of the Phacidiales. The hymenium arises in the same way, and the upper part of the stroma corresponds with the tissue which covers the hymenium before the opening of the apothecium. In the rose fungus, however, this covering is not so well developed and may not always persist to be folded back when the fruit body opens. It may form a continuous delicate layer over the asci until the fruit body is mature and only rupture together with the shield. In other cases this covering is broken by the development of the hymenial layer. Fragments may remain at the margin of the apothecium or they may disappear. It is only by the elongation of the asci and the consequent increase of pressure that the cuticle and shield, together with the upper part of the apothecium, are ruptured in an irregularly stellate manner and thrown back. The portions covering the hymenium have ruptured in fig. 11.

In the mature opened condition shown in fig. 12, the thin-walled cells of the upper part of the apothecium still persist on the margin of the fruit body. The opened perithecia present in surface view the appearance shown in fig. 5. The folding back of the shield is shown in section in fig. 15. The perithecia develop independently of the acervuli, as would be expected from the origin of the two. In fig. 13 is shown an old acervulus by the side of a perithecium. In none of the material which had wintered could acervuli be found which were bearing conidia.

The epidermal cells of the host persist for a long time, so that the ascogenous layer and shield are separated. They may become entirely destroyed as the asci elongate and the perithecium becomes mature (fig. 14), or they may persist on the margin of the mature perithecium (fig. 12). The perithecia vary in shape from spherical to discoid. One of the large discoid perithecia is represented in section in fig. 14. The septate, knobbed paraphyses extend between and beyond the asci until the time when the spores are nearly mature. Asci in many stages of development occur within each perithecium. Mature asci extend slightly beyond the paraphyses and the spores are discharged from an apical pore (fig. 16) formed by the rupture of the wall. The asci are oblong or subclavate, tapering above rather bluntly, and are $70-80 \times 15 \mu$.

Apparently the spores are not discharged with violence. Agar plates were inverted above rose leaves in moist chambers, the surface of the agar coming nearly in contact with the leaf. No spores were observed to have lodged on the surface of the agar, as would be expected if they were projected forcibly from the ascus. As far as I have been able to observe, they merely pile up in a whitish heap in the opened perithecium. The spores are $20-25 \times 5-6 \mu$, varying extremely in form (fig. 17), as do the conidia. They resemble the conidia very much except that they are not so strongly constricted at the septum. They are hyaline and bicellular. Usually large granules and several guttulae are present in each cell. The cells are generally unequal in size, the upper one being broader.

Germination of ascospores

Considerable difficulty was experienced in germinating the ascospores. All attempts to employ artificial media have been unsuccessful. Spores from the same preparation have been used in poured and planted plates of bean agar, in hanging drops of water, in similar drops in which has been placed a small piece of green rose leaf, in infusions made by boiling green rose leaves in water, and in drops of water on rose leaves in a moist chamber. In no case has germination been secured in any other way than by the last method. Germination occurs within 24 hours, the larger cell more often germinating, although either cell is capable of germination. A germ tube is characteristically formed at one side near the end of the spore. This hypha soon branches and septa are laid down (fig. 18). Occasionally two tubes are formed from a single cell. In about 35 transfers of spores to bean pods made under aseptic conditions no growth was secured. From these and the foregoing experiments it would seem that the ascospores are dependent on some stimulus of the living plant for germination. There may be some advantage to the parasite in this, since many spores would germinate before they are able to reach a suitable location on the host.

Artificial infection

Ascospores were used in the infection experiments. Since they are discharged in such masses in the opened perithecia, they can easily be removed free of everything else. Several series of poured plates made from spores obtained in this way remained absolutely sterile, which indicated that no other spores except ascospores of the rose fungus had been carried over. The spore masses were first removed to a drop of sterile water on a slide. With a needle, then, some of the spores were transferred to drops of water on the leaves of living roses. The plants were then covered with bell-jars and were allowed to remain covered for two days. Infections from inoculations made April 27 were very evident by May 7, appearing as small black areas. By May 15 mature acervuli and conidia of the *Actinonema* type were formed, thus completing the life cycle and connecting the two forms. Inoculations were

also made in the same way on leaves placed in Petri dishes lined with moist filter paper. In four days the radiating strands were very evident with the aid of the low power of a microscope. Infection occurs by the entrance of the germ tube through the cuticle, there being no stomata on the upper surface of the leaves. From the subcuticular mycelium, hyphae later penetrate to the tissue below, first filling the epidermal cells, and only in the advanced stages of the disease penetrating the mesophyll.

The way in which this fungus hibernates is no longer a matter of conjecture. SCRIBNER²¹ suggested that the spores lodge on the buds in autumn and remain there dormant until the leaves have expanded the following summer. As has been found to be true with many imperfect fungi, this fungus is carried through the winter on fallen leaves and the ascosporic stage develops in the following spring.

This study shows that *Gnomoniella rosae* (Fkl.) Sacc. is not the perfect stage of the rose *Actinonema*, as has recently been suggested.²² One species of *Actinonema*, however, has been connected with an *Asterella*, *Actinonema rubi* (Fkl.) becoming *Asterella rubi* (Fkl.) v. Höhnelt.²³ He found in the spring the *Asterella* stage on living canes of *Rubus Idaeus*. These areas had been occupied the previous summer by the conidial stage.

The genus *Asterella* was first proposed by SACCARDO²⁴ as a subgenus of *Asterina* for those species which have hyaline spores. Later ²⁵ he raised *Asterella* to generic rank. As SACCARDO himself points out, further investigation of species which are at present placed in the genus *Asterella* will result in their transfer to *Asterina*, since the spores become brown at maturity. LINDAU²⁶ thinks the existence of this genus is still questionable. Subsequently but little investigation has been made on the genus and no clear-cut

²¹ See footnote 7.

²² LAUBERT, R., and SCHWARTZ, MARTIN, *Rosenkrankheiten und Rosenfeinde*. 16-19. 1910.

²³ VON HÖHNEL, F., Über *Actinonema rubi* Fuckel ist *Asterella rubi* (Fkl.) v. Höhnelt. *Ann. Myc.* 3:326. 1905.

²⁴ SACCARDO, P. A., *Syll. Fung.* 9:393. 1891.

²⁵ ———, *Syll. Fung.* 1:25 and 42. 1882.

²⁶ LINDAU, G., ENGLER and PRANTL's *Pflanzfam.* 1:340. 1897.

generic limits have been proposed. One finds included species whose spores become brown, some which are aparaphysate, some possessing filiform paraphyses, and others having paraphyses which are enlarged at the tips. In fact, the whole family Microthyriaceae is but little known, due in part to the fact that most of the forms are tropical. A thorough investigation of perithecial development is necessary, since very little attention has been given to this group. The family is at present characterized by having perithecia which are shield-shaped, thin membranaceous, flat, with a rounded pore at the top and with a membrane formed *only on the upper side*. With the exception of the species on rose leaves, which I have studied, it is not known whether or not the forms without an apical pore possess one at maturity. It has long been recognized, because of the entirely different manner of development, that the Microthyriaceae are widely separated from the other two families of the Perisporiales, the Erysiphaceae and the Perisporiaceae.

In order to see if other genera of the Microthyriaceae corresponded in structure and development with the forms on rose leaves several of them were examined. *Asterina orbicularis* B. & C., n. 231 of RAVENEL'S collections, forms entirely superficial perithecia, sending hyphae partially through the cuticle. *Asterina inquinans* E. & E., n. 1785 N.A.F., is also superficial, ends of the mycelium being observed in the stomata. *Asterina plantaginis* Ellis, n. 791 N.A.F., forms spherical perithecia entirely sunken within the host tissue. The perithecia are ostiolate and appear to have the characters of a *Sphaerella*. *Micropeltis longispora* Earle, n. 6349, plants of Porto Rico, is entirely superficial. *Microthyrium littigiosum* Sacc., collected at Frankfort, Germany, by Dr. PAUL MAGNUS, seems to form superficial perithecia, but the mycelium is present in the epidermal cells. *Myriocopron smilacis* (De Not.) Sacc., n. 600 E. & E., N.A.F., also forms superficial perithecia and the mycelium occurs in the stomata. None of these representative genera seem to be comparable to the type of development as exhibited by the rose fungus. Since so little is known of the perithecial development and the method of securing food supply of the Microthyriaceae, this family would afford an

excellent field for investigation. MAIRE²⁷ has described the organs of absorption of *Asterina usterii* and *Asterina typhospora*. A slender filament penetrates the epidermal wall and when it has reached the cavity of the cell it enlarges and becomes profusely branched.

The opened perithecia of the rose fungus present characters indicative of a close relationship to the Phacidiales. The ragged margin of the shield suggests the ruptured outer portion of the wall of the fruit body which at first covers the hymenium. The presence of knobbed paraphyses is also a character possessed by many Discomycetes. In the Phacidiales, however, as far as can be learned, the upper or outer part of the fruit body is not separate in origin from the ascogenous stroma, nor does it possess the characteristic structure, of the shield present in the Microthyriaceae. On the other hand, it is quite probable that few of the Microthyriaceae possess a stroma within the leaf tissue as has been described for the fungus in question. The majority are apparently superficial and with a well developed wall or shield *only on the upper side*. In spite of these facts, I feel that this fungus should be placed in the Microthyriaceae. Further morphological study of other species of this genus and related genera will throw some light on the relationship of these microthyriaceous forms. Perhaps the systematic position of many of these forms will be changed as soon as the species have been satisfactorily investigated. While the possession of the shield and the hyaline 2-celled spores are characteristics which would suggest the position of the rose fungus in the genus *Asterella*, yet, as has been pointed out, this genus is not clearly limited and contains heterogeneous elements. This fungus does not seem to accord morphologically with the members of this genus in the sense in which the genus was first employed. Species representing several generic types apparently have been included in *Asterella*. Since the characters presented by this fungus are evidently those of a distinct generic type, rather than place it in the genus *Asterella*, it seems better to treat it as the type of a new genus. Because of the two separate structures, the shield

²⁷ MAIRE, R., Les Suçoirs des *Meliola* et des *Asterina*. Ann. Myc. 6:124-128. fig. 4. 1908.

and apothecium, the name *Diplocarpon*²⁸ is proposed. The following description of the genus is given.

Diplocarpon, nov. gen.—Fruit bodies formed in connection with an extensive subcuticular mycelium, consisting of a subcuticular circular shield with more or less radiate elements especially at the margin, and an innate apothecium. Shield, together with the radiating strands on which it is formed, dark brown, without a central pore. Apothecium at first separate from the shield, only joined here and there by hyphae which pass between the epidermal cells. Apothecium joined with the margin of the shield at maturity. Hymenium covered by the shield and upper part of the apothecium which at maturity rupture in an irregularly stellate manner. Asci oblong to subclavate, 8-spored; paraphyses unbranched; spores elongated, 2-celled, hyaline at maturity.

A conidial stage of the *Actinonema*-type occurs in one species.

Perithecii scutulum subcutaneum et apothecium innatum constitutis; scutulo mycelio subcutaneo, lato extenso, atro-brunneolo insidente; margine radialiter diffuso, contextu membraneo, astomate; apothecio innato, primo scutulo separato, maturitate margine adjuncto. Perithecii centro stellatim laciniato-dehiscentibus; ascis oblongis; paraphysibus simplicibus; sporidiis oblongo-ellipticis, bicellularibus, maturitate hyalinis.

Actinonema uni speciei cujus statum conidicum sistit.

Since this study connects for the first time the ascosporic stage with the conidial stage of the black spot of rose leaves, a brief characterization of the species is added:

Diplocarpon rosae, n.n.

Syn. *Erysiphe radiosum* Fr. *Observationes Mycologicae*. 207. 1824. *Asteroma rosae* Lib. *Mem. Soc. Linn.* 5:404-406. 1827. *Actinonema rosae* Fr. *Summa. veg. Scand.* 424. 1849. *Dicoccum rosae* Bon. *Bot. Zeit.* 282. 1853. *Marsonia rosae* Trail. *Fung. Inverar.* 46. 1889. *Marsonia rosae* Br. and Cav. *Funghi parassiti* n. 97. 1889.

Ascigerous stage.—Perithecia epiphyllous, spherical to disciform, 100–250 μ in diameter; upper part or shield dark brown, subcuticular, formed in conjunction with the radiating strands of mycelium, circular, with a more or less radiating structure toward the margin. Lower part of fruit body disciform, subepidermal, of several layers of pseudoparenchyma cells, the outer of which are dark brown,

²⁸ διπλός, "double," καρπός, "fruit."

the margin at length breaking through the epidermis and here and there becoming connected with the margin of the shield. Fruit bodies closed at first, later opening by the rupturing of the shield together with the upper part of the apothecium in an irregularly stellate manner from the center. Asci oblong or subclavate, narrowed abruptly above, $70-80 \times 15 \mu$, 8-spored; paraphyses slender, enlarged abruptly at the tip, often 1-septate. Spores oblong-elliptical, hyaline, unequally 2-celled, constricted at the septum, $20-25 \times 5-6 \mu$; upper cell somewhat larger, cells usually guttulate.

Conidial stage.—Spots epiphyllous, large, dark brown or blackish, with an irregular radiating border, when numerous becoming confluent and sometimes involving the entire leaf. Mycelial strands composed of several filaments, at first hyaline, forming a subcuticular network. Internal mycelium connected through the epidermis with subcuticular mycelium. Acervuli subcutaneous, covered at first by the cuticle which ruptures irregularly; conidia 2-celled, often deeply constricted, straight or subfalcate, $18-25 \times 5-6 \mu$, hyaline and guttulate.

Conidial stage appearing on rose leaves in summer and autumn often causing defoliation of the plants. Ascigerous stage appearing in the spring on fallen leaves which have remained on the ground.

Peritheciis epiphyllis, globosis v. disciformibus, $100-250 \mu$ diam.; scutulo atro-brunneo, subcutaneo, mycelio reticulato insidente, orbiculare, margine plus minusve radio. Apothecio primo epidermide tecto, demum margine scutuli adjuncto, in centro irregulari-stellato dehiscente. Ascis oblongis vel subclavatis, supra obtuse angustatis, $70-80 \times 15 \mu$, octosporis; paraphysibus filiformibus, apice incrassatis, interdum 1-septatis; sporidiis oblongo-ellipticis, inaequaliter bicellularibus, ad septa constrictis, guttulatis, hyalinis, $20-25 \times 5-6 \mu$.

Hab. in foliis dejectis Rosae sp.

Status conidicus: Maculis epiphyllis, atro-brunneis vel purpurascensibus, fibrillis e centro radiantibus, albido-arachnoideis; acervulis subcutaneis, sparsis, nigricantibus; conidiis constricto, 1-septatis, guttulatis, hyalinis, $18-25 \times 5-6 \mu$.

Hab. in foliis vivis Rosae sp.

Susceptibility of the host

This disease occurs on nearly all the cultivated varieties of roses both out of doors and in the greenhouse. BRIOSI and CAVARA note that only four varieties, *Rosa hybrida* var. Belle

Angevine, Triomphe d'Alençon, Abel Grant, *Rosa borboniaria* var. Triomphe d'Anger, of the 600 growing in the botanical gardens at Pavia are free from the attacks of this fungus. LAUBERT and SCHWARTZ²⁹ call attention to the fact that the bushy sorts are more susceptible than climbing varieties, and also that thin-leaved species are most liable to attack. HALSTED³⁰ finds that a wild species, *Rosa humilis*, is also subject to attack when growing in a garden with diseased plants. The amount of loss caused is equaled or surpassed by only one other rose disease, the powdery mildew.

Control measures

This disease has been very satisfactorily controlled by the use of any of the standard copper compounds. Since now we know that the fungus winters over in the fallen leaves, sanitary measures may better be employed in combating the disease. If all the leaves are gathered together and burned either late in the autumn or early in the spring, before the new leaves have expanded, the chances of infection would be greatly lessened.

This investigation was undertaken at the suggestion of and under the careful direction of Professor GEORGE F. ATKINSON, Cornell University, to whom I am very grateful for help and criticism.

AGRICULTURAL EXPERIMENT STATION
AUBURN, ALABAMA

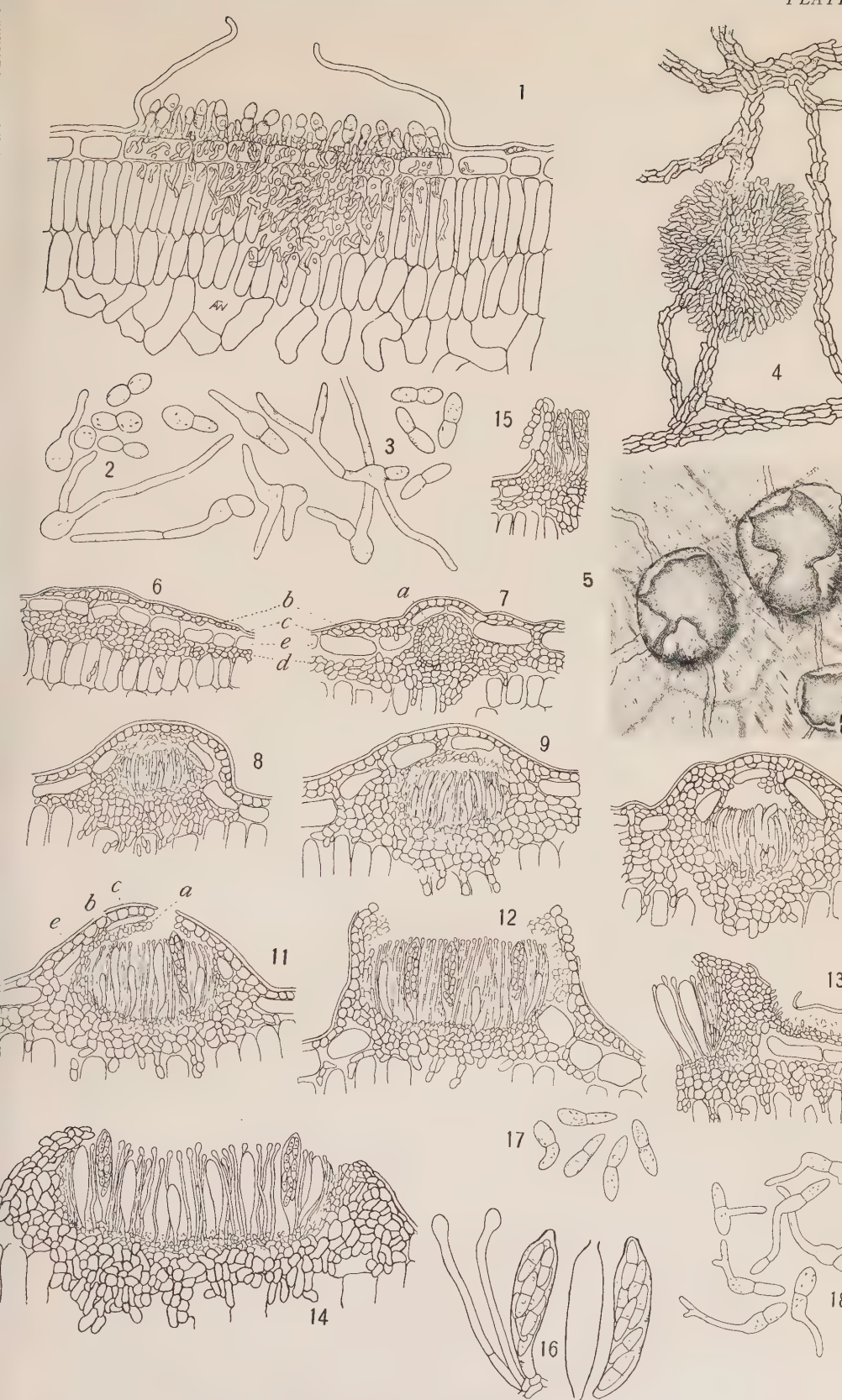
NOTE.—Since this manuscript has been sent to the publishers, I have received type specimens of *Asterella rubi*, which had been sent to Professor GEORGE F. ATKINSON, through the courtesy of Professor F. VON HÖHNEL. Because of the fact that *Asterella rubi* is the first *Asterella* to be connected with an *Actinonema*, and is one of the most recently described species of this genus, it is especially important that it be compared morphologically, with the rose fungus.

For the study of the structure of the fruit bodies of *Asterella rubi* the cortex of some of the affected raspberry canes was imbedded in paraffin and sectioned. The perithecia were found to possess a central pore or ostium. They are entirely superficial and with a well developed structure only on the upper side. There is no well defined stroma from which the asci arise.

By treating small pieces of the cortex with lactic acid the entire shield may

²⁹ See footnote 22.

³⁰ HALSTED, B. D., New Jersey Agr. Exp. Sta. Rept. 13:281. (1892) 1893.



WOLF on ACTINONEMA ROSAE

be loosened, and can be floated away, thus proving beyond a doubt that this structure is wholly superficial and not subcuticular. *Asterella rubi*, therefore, conforms to the present concept of the genus *Asterella*, but is of an entirely different generic type from that represented by *Diplocarpon rosae*.

EXPLANATION OF PLATE XIII

FIG. 1.—Acervulus of conidial stage (*Actinonema rosae*), with a section of one of the radiating strands at the right of the acervulus; $\times 400$.

FIG. 2.—Conidia formed free in culture; the two halves are easily separable; germination of the separated cells; $\times 400$.

FIG. 3.—Normal conidia from acervuli, and their method of germination; $\times 400$.

FIG. 4.—Surface view of *Diplocarpon rosae*, showing the shield and subcuticular strands from which it developed; $\times 110$.

FIG. 5.—Surface view of mature perithecia in which the shield has been ruptured irregularly and folded back; $\times 55$.

FIG. 6.—A very young stage in the development of a fruit body in which the shield and the stroma from which the asci are formed are distinct; C, cuticle; B, epidermal cells; D, ascogenous stroma; $\times 200$.

FIG. 7.—A stage in which the fruiting part of the perithecium has begun to be differentiated; the shield and ascogenous stroma are separate; A, young apothecium; $\times 200$.

FIG. 8.—Differentiation of the asci within the apothecium; $\times 200$.

FIG. 9.—Perithecium in which the epidermal cells still persist between the apothecium and shield; the thin-walled cells of the upper part of the apothecium form a covering over the hymenium; $\times 200$.

FIG. 10.—Perithecium in about the same stage of development as fig. 9, but the shield and fertile stroma are completely united; $\times 200$.

FIG. 11.—Perithecium which is nearly mature; the hymenial covering has broken; A, upper part of apothecium; B, shield; C, cuticle; E, epidermal cells; $\times 200$.

FIG. 12.—Mature fruit body of *Diplocarpon rosae*; some of the cells of the upper part of the apothecium persist at the margin; $\times 200$.

FIG. 13.—An old acervulus persisting at the side of a perithecium; $\times 200$.

FIG. 14.—A large disciform perithecium; some asci are mature and in others the spores have not yet been formed; the ascogenous stroma and shield have grown together and the epidermal cells have been destroyed; $\times 200$.

FIG. 15.—Section through a mature perithecium, showing the manner in which the shield is folded back; $\times 200$.

FIG. 16.—Mature asci and paraphyses; the spores have been discharged apically from one ascus; $\times 400$.

FIG. 17.—Ascospores of *Diplocarpon rosae*; $\times 400$.

FIG. 18.—Germination of ascospores; $\times 400$.

Some Fungous Diseases of the Prickly Pear, *Opuntia Lindheimeri*† Engelm.¹⁾.

By Fred. A. Wolf.

(With Plates I—III.)

During the past three years some of the most noticeable and most common diseases of the prickly pear have been made the subject of a special study. This work has been greatly facilitated by the fact that material was so easily available. The region in which Austin, Texas, is located, is very favorable, especially as regards moisture and temperature, for the growth of the prickly pear. Under the semi-arid conditions which prevail throughout this region the prickly pear thrives abundantly, not because of these climatic conditions, however, but because it is naturally so well adapted to extremes of heat and drought. It is indigenous to the southern and southwestern portions of the State, forming often dense, impenetrable thickets. It may occur alone or associated with mesquite (*Prosopis glandulosa*), or other leguminous shrubs or even with oaks (*Quercus* sps.) occurring in the open spaces between the trees or forming with them dense impenetrable thickets (Fig. 1).

Within recent years, under the direction of the United States Department of Agriculture^{2) 3) 4) 5)}, some practical investigation of cacti has been accomplished showing that there are many unemployed possibilities in cactus culture. Their economic value as a forage crop has been determined both by chemical analyses and by feeding experiments.

† Several other species of platyopuntias occur in this locality and are affected by the same organisms.

1) Contribution from the Department of Botany, Cornell University, No. 144.

2) Griffiths, D. The Prickly Pear and Other Cacti as Food for Stock. U. S. D. A. Bur. of Plant Industry, Bull. 74: 1—48. Pl. I—V. 1905.

3) Griffiths, D. Feeding Prickly Pear to Stock in Texas. U. S. D. A. Bur. of Animal Industry. Bull. 91: 1—23. Pl. I—III. 1906.

4) Griffiths, D. and Hare, R. F. The Tuna as Food for Man. U. S. D. A. Bur. of Plant Industry. Bull. 116: 1—66. Pl. I—II. 1907.

5) Griffiths, D. The Prickly Pear as a Farm Crop. U. S. D. A. Bur. of Plant Industry. Bull. 124: 1—32. Pl. I—II. 1908.

When fed with more concentrated feeds they may be very profitably employed as roughage. Cacti have long been used, however, as forage, yet it is only in times of drought when other food is scarce. The practicability of their cultivation as a forage crop when grown under xerophytic conditions has been considered and they are found to respond very favorably to cultivation.

Along with the growing recognition of the economic value of the pear will come the consideration of its natural enemies. From time to



Fig. 1.

time there has appeared in mycological literature brief descriptive notes of the various fungous enemies. While some of them are quite serious enemies of this plant, no special consideration has hitherto been given to a detailed study of any of them. Among those which have been described as occurring on cacti in North America are the following:

Phytophthora cactorum (C. & L.) Schroet. Beit. Biol. Pflanz. **1**: 51—57, 1870.

Teichospora opuntiae E. & E. on *Opuntia arborescens*. Colo. Bull. Torr. Bot. Club. **24**: 459, 1897.

Rhytisma cacti Schw. on *Opuntia* sp. Mass. Syn. Amer. bor. no. 2045.

- Gloeosporium opuntiae* E. & E. on *Opuntia brasiliensis*. Mo. Jour. Myc. **4**: 52, 1888.
- Gloeosporium cactorum* Stonem. on *Opuntia* sp. N. Y. Bot. Gaz. **22**: 82, pl. VII, fig. 14, 1898.
- Lembosia cactorum* Tracy & Earle on *Opuntia vulgaris*. Fla. Bull. Torr. Bot. Club. **28**: 186, 1901.
- Nectriella cacti* E. & E. on *Opuntia ficus-indica*. Ala. Jour. Myc. **8**: 66, 1902.
- Gloeosporium lunatum* E. & E. on *Opuntia* sp. La. Proc. Acad. Phil. **43**: 82, 1891.
- Sphaerella opuntiae* E. & E. on *Opuntia* sp. Tex. Jour. Myc. **4**: 97, 1888.
- Perisporium Wrightii* B. & C. on *Opuntia macrorrhiza*. Tex. Cuban Fungi no. 987.
- Hendersonia opuntiae* E. & E. on *Opuntia ficus-indica*. Ala. Jour. Myc. **8**: 65, 1902.

The last four fungi included in this list are prevalent in the immediate vicinity of Austin and will receive further consideration.

Purpose and Method of Work.

It is intended in this work to consider not only the structure, cultural characters and life history of the fungi themselves but also symptomology and the pathological morphology of the pear. This necessitated that the field study made during the season of the plant's greatest activity, be supplemented by laboratory study. In order to have plants where absolute control measures might be employed in inoculation experiments, some of the segments were planted in the green house.

In order to avoid the danger of rotting the segments were permitted to dry for some time before being partially buried in the earth. The plant establishes itself very readily, the roots arising from the areolae of the spines. Since the segments resist drying out for a very considerable time, they could be kept in moist chambers in the laboratory. Where it was desired to keep them longer, good results were obtained by sealing the end injured by the separation from the adjacent segment, with melted paraffin.

Material for microtoming was fixed in several of the common solutions, Flemming's medium, Gilson's, Picric Acid and Chromo-acetic acid. Gilson's fluid is known to give very satisfactory results especially where it is desired to preserve the conidia in position in acervuli or sporodochia. Because of the presence of a thick hard cuticle and the abundance of calcium oxalate crystals both in the first hypodermal layer and scattered within the mesophyll of the segment, great difficulty was experienced in cutting suitable sections.

When the fungi had progressed so that the tissue was badly disintegrated it could be quite readily sectioned. In much of the work free

hand sections could be made which were very serviceable. This work was begun at the suggestion of Dr. F. D. Heald at the University of Texas to whom I wish to acknowledge my thanks for advice and help. It has been completed at Cornell University under the direction of Prof. George F. Atkinson to whom I wish to express my gratitude for help and kindly criticism.

Anthracnose of the Prickly Pear.

This disease is produced by a fungus, *Gloeosporium lunatum* E. & E.¹⁾ which was described as occurring on living *Opuntia*²⁾ from San Antonio, Texas. It is so commonly present in the Southwestern United States that it is the exception to find a plant entirely free from the disease.

Signs or Symptomology.

Plants which are attacked by this fungus show circular or sub-circular depressed grayish areas, densely dotted on both surfaces with minute black fruit-bodies which do not cover the entire spot (Fig. 13). Quite commonly a zone of brown marks the border of the anthracnosed area. The disease first manifests itself by the formation of soft sunken brownish areas which after a few days are covered with an abundance of flesh colored heaps, the acervuli. Within a few weeks these areas have become hard and dry and the acervuli have blackened. The spots are generally 1.5—2.5 cm in diameter. Isolated spots may coalesce causing the formation of larger spots or as frequently occurs by this fusion, the destruction of the entire segment.

Normally the diseased areas extend entirely through the stem segment. This dead tissue may persist, or as frequently happens, may fall out leaving a clear circular opening through the joint (Fig. 10). Any portion of the segment may be attacked, so that in case the spot is marginal, a notch may be formed, or when central a perforation, as has been described.

The fruits of the pear are also attacked resulting in their complete destruction or the formation of anthracnosed spots (Fig. 11). Sometimes only a single diseased spot is formed on a segment. In other cases the spots may be very numerous. In this crowded condition the fungus is able to form only pox-like depressions extending partially through the segment (Fig. 12). Such spots usually become dry before conidia have been formed. When the infections occur late or on rather more mature segments the formation of conidia is entirely inhibited (Fig. 9).

¹⁾ Ellis, J. B. and Everhart, B. M. Proc. Acad. Phil. 43: 82. 1891.

²⁾ The three species of *Gloeosporium* which have been described as occurring on *Opuntia* in the United States are easily separated. *Gloeosporium lunatum* differs from *Gloeosporium opuntiae* in its maculiculous growth and lunate conidia. It differs from *Gloeosporium cactorum* in that it does not form a close ring of acervuli, has entirely different cultural characters and conidia of a different size.

This fungus produces on young segments under favorable conditions of temperature and moisture a severe rotting which may advance through an entire segment in a few days starting from a single center of infection. Frequently a gummy exudation accompanies these spots. When the diseased spot is formed at the point of attachment, the segment drops off. Plants have been observed in the field in which nearly all the new segments succumbed in this way.

The symptomology for this disease is the same as has been described by Griffiths (4.), the cause of which he attributes to the black spot fungus, *Perisporium Wrightii*¹).

While he gives no illustrations of the fungus, yet from the description, as given, there can be no doubt that he is dealing with symptoms characteristic of this anthracnose.

The Acervuli.

Because of the difficulty experienced in making sections suitable for determining the structure of the acervulus it was found advantageous to permit the tissue to become thoroughly disintegrated by keeping the segments in a moist chamber. Acervuli on young and old segments taken directly from the field or having been kept in a moist chamber, exhibited the same structure, the difference being merely in the fact that it was practically impossible to section the material taken directly from the field. The conidia remained in position upon the conidiophores when Gilson's fixing solution was used.

The acervuli arise from a pseudoparenchymatous layer beneath the epidermis. As this fungous tissue increases certain of the hyphae are directed perpendicularly to the surface (Fig. 15). By their upward growth and the consequent increased pressure, the epidermis is ruptured. The direction of growth continues the same and a cushion-like stroma is formed. This stroma is often almost entirely above the surface of the segment. The acervuli most frequently form within the stomata (Fig. 16). They are 60—125 μ in diameter and are at first salmon colored, the color being imparted by the conidia. As they become older and cease to form conidia, they become brown and then almost black with perhaps a faint tinge of

¹) The black spot fungus, *Perisporium Wrightii*, is first found as a soft brownish area usually more or less circular on any portion of the joint. This soon becomes black and rotten, and later dries up when the ascogenous bodies appear upon the epidermis. Often there may be a dozen of these spots on a single joint and they are approximately $\frac{1}{2}$ to 1 inch in diameter, usually, and extend clear through the joint. When but one or two occur the joint may recover, the tissue healing up around the diseased portion, when the dried diseased tissue falls out leaving a notch or a clear circular opening through the joint. When the diseased spots are numerous the plants are very much impoverished and the joints often drop off, the healing in this case occurring at the union between them.

gray. The conidia are lunate-fusoid, $12-20 \approx 2-3 \mu$ and for the most part 1-septate (Fig. 17). Those formed on the acervuli are more or less attenuated at each end, young and immature conidia being rounded. In culture the conidia are never acute but always bluntly rounded and generally one-celled (Fig. 19).

As will be readily seen this type of acervulus and conidium does not conform to the general concept of the genus *Gloeosporium*. In fact it was long a matter of doubt in the writer's mind as to whether or not it should be considered a member of the Melanconiaceae, since the character of the acervulus suggests that it properly belongs among the Tuberculariaceae. There appear to be several good reasons for retaining this fungus in the genus *Gloeosporium*. As is well known, it is sometimes very difficult to decide, even after the structure and relationship of parts is known, in which family an organism belongs. Certain species present characters of two families and seem to be intermediate between them.

Two genera of the Melanconiaceae, *Coryneum* and *Hainesia*, develop quite prominently projecting stromata. While the acervuli of the greater number of species of *Gloeosporium* are scarcely elevated above the surface of the host tissue or are somewhat subdisciform, the generic limits permit the inclusion of pulvinate or cushion-like forms. *Gloeosporium lunatum* might well be considered as one of the extremes in the formation of a prominently projecting stroma upon which the short conidiophores and conidia are borne. This formation may be due in part to the fact that the hypoderma is more or less rigid by the time that the acervuli are being formed. As the stroma which is to become the acervulus is forming in the epidermal cells, the only chance for expansion is upward, the tissue beneath being but little or not at all pliant. As a result the acervulus is for the most part superficial and protruding. This argument loses weight in the case of *Gloeosporium opuntiae* E. & E.¹⁾ on *Opuntia brasiliensis* where the collenchymatous hypoderma does not prevent the formation of a typical acervulus of *Gloeosporium*. No evidence is at hand in this form though as to the age at which the stems are attacked, dry material only being examined.

As to the septation of the conidia it might be noted that at present there are included in *Gloeosporium* several species which contain occasional conidia which are septate. *G. decipiens* E. & E., *G. orni* Sacc. and *G. victoriense* D. Sacc. approach *Marsonia* while *G. rostratum* E. & E. approaches *Septogloeum*. The main reason for retaining it in *Gloeosporium* is that it presents when examined with the unaided eye all the appearance of a *Gloeosporium*. The typical anthracnosed area with heaps of pinkish acervuli and conidia immediately suggest the characters of a *Gloeosporium*.

¹⁾ This material was obtained through the courtesy of the New York Botanical Garden.

Germination of conidia.

Upon germination the conidium contents become granular and a large vacuole is generally present. The germ tube grows out at one end. It is a common phenomenon for a tube to be formed at each end of the two-celled spores (Fig. 18). When the medium is not suitable the spore may become four-celled before the formation of the germ tubes. The hyphae soon become irregularly septate and much branched. The conidiophores are often formed very near to the mother conidium. At first a slender finger-like projection appears on the side of the hypha. This enlarges at the distal end and eventually is constricted and entirely separated from the short conidiophore. By the successive acrogenous abjunction of conidia small heaps are piled up. There is no aggregation of these conidiophores into acervuli in cultures but the conidiophores and conidia arise from any part of the mycelium (Fig. 19).

Character of the colony.

In all the various kinds of media employed, the conidia were found to germinate readily. On glucose agar the growth is rapid forming a colony with a yellowish brown tinge. The colonies are whitish at first but in the course of a few days they become darker. The mycelial branches radiate from a central portion which is quite compact giving to the margin of the colony an arborescent appearance. The mycelium grows very close to the substratum not rising perceptibly above the agar, so that the whole colony has a smooth, watery look. When the cultures are grown where they are exposed to intense diffuse light during the day, concentric zones mark the daily growth. On corn meal the mycelium is at first whitish and loose, indicating the rapid growth. This color is soon changed to a very pronounced violet.

Time of Infection.

There is a definite seasonal period in which infection occurs. This period is always in the spring and is entirely controlled by two factors, the first of which is climatic and the second dependent on the age of the segment. In the vicinity of Austin there was in the year 1909, a rainy and cloudy period of about a week's duration during the latter part of March. Immediately following this there was an abundance of the anthracnose. In the following year the rainy season did not come until a month later to be again followed by a severe diseased condition. During the two weeks preceding May 5th, 1911, it was continuously cloudy and rainy. The first infections were so abundant that the segments were destroyed while those coming a few days later produced the characteristic spots. Under normal conditions the pear contains enough reserve food material to start the growth of the new joints. The rainy season comes at a time when this new growth is well started.

No new infection has been observed to occur in the field on old segments. It is only before any marked cuticularization or differentiation of the underlying tissues has taken place that infection may occur. The growth of the pear is so rapid that unless infection occurs in these few favorable rainy, cloudy days, it probably does not occur at all.

Method of Infection.

New crops of conidia may be obtained from old diseased areas at any season of the year if the proper conditions of moisture and temperature are supplied. If segments are placed in moist chambers, new acervuli are formed on the old diseased areas. The mycelium may spread through the unaffected part of the segment and after the disintegration of the tissue, the new acervuli appear on the surface. Cultures were also obtained at various times throughout the year by plantings of the dried tissue from infected areas. All of this shows that the mycelium remains viable in the diseased spots through the entire year. Repeated attempts were made to produce infection on plants in the green house and laboratory both by placing a portion of the mycelium on the surface of the segment and by spraying on the conidia with an atomizer. In the green house some of the plants were covered with bell jars for a few days and others left uncovered. Those in the laboratory were kept in moist chambers. In no case by this method of inoculation could infection be secured. Segments of all ages were tried varying from those which were just emerging to those which were fully developed. If, however, a wound was made either with a needle or a scalpel and some of the conidia were placed in the opening, infection was sure to follow. In young segments the growth of the fungus within the tissues is very rapid. Areas three-quarters of an inch in diameter and extending clear through the segment were produced within seventy-two hours after inoculation. Segments of the previous year's growth were wounded and inoculated. Infection would sometimes occur but growth was slow and would not advance far. Only a small depression of dead tissue would be formed and the areas never extended through the segment. Old segments have been observed in the field, however, during the rainy season, in which the fungus had spread from the primary center of infection and destroyed the remainder of the segment. This very commonly happens when segments are kept in a moist chamber.

At the time when infection occurs in the field, a bug, *Chelinidia vittigera* Uhler¹⁾ is very common upon the segments. This bug punctures the segments in securing its food. By so doing it is probably responsible for at least part of the infection. Direct evidence on this point is enti-

¹⁾ For this determination thanks are due to Prof. A. D. Mac Gillivray, Cornell University.

rely negative, however. Bugs were permitted to feed on healthy, young segments enclosed under the same bell jar covering segments with diseased areas bearing conidia. As far as could be observed they did not carry the conidia over to the healthy joints but from their sucking habit it would seem quite likely that they might produce the wound through which infection might take place.

Effect on the Host.

The growth of this fungus is very rapid occupying about a week in the formation of the anthracnosed area. The infected tissue is for the most part completely destroyed. The cuticle, epidermis and hypoderma are quite resistant, while the chlorenchyma is wholly disintegrated.

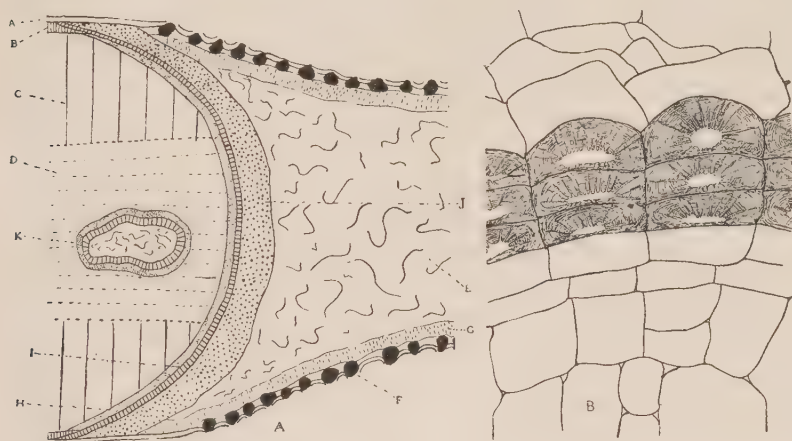


Fig. 2.

Fig. 2 A represents diagrammatically the position of the tissues in an anthracnosed area and its relation to the normal tissue. A layer of parenchyma cells (H.) becomes differentiated a little distance in advance of the fungus which is spreading through the tissues. The cells of this layer are uniform in size and are changed into collenchyma, (I.), forming a layer 3—5 cells thick which is continuous with the collenchymatous hypoderma. In this way the margin of the wound is perfectly healed. Some of these collenchyma cells and the cells from which they are formed are shown in Fig. 2 B. This does not appear to be a common method of healing wounds in other plants. According to de Bary¹⁾ wound surfaces of whatever sort are closed and healed by the formation of a layer of cork.

¹⁾ DeBary, A. Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns, 108, 1884.

In order to see whether the formation of this layer was due to any stimulative action by the fungus, holes were cut through active segments. In a short time the wounded surface had been perfectly healed by the formation of collenchyma tissue, showing that the response is made to the presence of wounds regardless of the cause.

Often the fungus breaks through the barrier or has already passed beyond the place of the formation of these thick-walled cells before they are sufficiently developed. In this case the attempt to check the advance of the fungus is repeated and an isolated area, entirely or for the most part surrounded by collenchyma, is formed (Fig. 2 K.).

Development of Perithecia.

The ascigerous stage of this fungus was described by Ellis and Everhart¹⁾, in 1888, as *Sphaerella opuntiae* E. & E. on dead *Opuntia* from Louisiana²⁾. Three years later they described the conidial stage as *Gloeosporium lunatum* noting the fact that it was associated with *Sphaerella opuntiae*. They did not, however, suggest a possible relationship between these forms.

During the three years in which this fungus has been cultured from conidia, perithecia have never appeared in these cultures. Glucose agar, glycerine agar, lactose agar, bean agar, gelatine, bean stems, bean pods, corn meal, and sterilized pieces of cacti have been used as culture media. In the field, through, the ascigerous stage invariably follows the conidial stage. This constant association suggested the idea of a possible connection. Apparently mature perithecia have been found in the field on segments about a month after the formation of the anthracnosed areas. The perithecia appear on both surfaces of the spot as densely crowded, black bodies. They arise from the stromata left by the old acervuli. The stroma of the acervulus is at first hyaline and when conidia are no longer being produced the fungous tissue becomes dark brown and the cell walls thicken. This gives to the stroma a black color. As it increases in size it pushes outward and at the same time extends downward into the stomatal chimney (Fig. 20). By the increased growth of this fungous tissue the partially disintegrated hypodermal cells bordering the substomatal cavity are crowded apart (Fig. 21). These cells become more and more disintegrated as growth continues. Often the stromata push the cuticle and outer epidermal wall upward so that the perithecial cavity is for the most part above the crystal-bearing hypoderma (Fig. 22). In this figure is also shown an early stage preparatory to the formation of asci. Certain of the cells of this stroma lose their brown color, become thinner walled forming a flask shaped receptacle in which the asci are

¹⁾ Ellis, J. B., and Everhart, B. M. Jour. Myc. 4: 82, 1888.

²⁾ The type material which I was able to examine through the courtesy of the New York Botanical Garden is the same as the material studied from Texas.

formed. The wall of the perithecium is 50—100 μ in thickness. A short stout beak projects beyond the stromatic surface marking the position of the ostiolum. The cavity is 100—120 μ in diameter (Fig. 23). Arising from the base are the elongated asci, $60 \approx 12$ —15 μ , thickened apically, with a pore in the thickened part (Fig. 24). The spores are hyaline 20—22 $\approx$ 3,5—4 μ and 1-septate (Fig. 25). They are guttulate, slightly incurved at the septum and typically arranged in two rows. All attempts to germinate these ascospores during the summer, autumn and early winter were unsuccessful. However, in a plate poured Febr. 15th a few spores of the several hundred in the plate were observed to have germinated (Fig. 26). Colonies of *Gloeosporium* developed in these plates, whether from ascospores or from bits of mycelium could not be determined. The time at which germination was secured, however, would seem to correspond with what one would expect in the field since infections occur late in March or early in April. Several attempts were made to isolate the fungus from single germinating spores, but so few germinated that colonies, developing from spores whose position had been marked on the bottom of the petri dishes, were never observed. The segments from which these spores were obtained had been kept in the laboratory since the previous June, and the spores appeared not to have developed fully. In a collection of several dozen diseased segments made in the spring of 1911 no mature perithecia could be found due perhaps to the fact that the previous summer had been unusually hot and dry. For this reason the development of the conidial stage in cultures from germinating ascospores was not successful. Even though the perithecia did not mature there was an abundant infection in 1911 due to the fact that the mycelium remains viable in the old diseased areas.

The type of perithecium possessed by this fungus does not agree with that which is characteristic of the genus *Sphaerella*. *Sphaerella* possesses a perithecium with a thin, membranaceous wall, and the ostiolum is plain or slightly papillate. In the genus *Gnomonia* the wall of the perithecium is leathery or submembranaceous and rather thick. The ostiolum is rostrate. The wall of the ascus is thickened with an apical perforation in the inner wall. Edgerton¹⁾ has pointed out, however, that even in the same species, the neck of the perithecium may be long or entirely wanting. *Gnomonia veneta* on the oak has a long neck while on the sycamore the neck is very short or entirely absent. No differentiated neck is developed in the form on *Opuntia*, but the ostiolum is formed by the outward projection of the perithecial cavity. The fruit body does not appear to be a true perithecium, but rather presents the character of a stroma with a single perithecial cavity. This structure is certainly

¹⁾ Edgerton, C. W. The Physiology and Development of Some Anthrac-noses. Bot. Gaz. 45: 367—408. Pl. XI, f. 17. 1908.

very different from the perithecial wall of either *Sphaerella* or *Gnomonia*. Its relation appears rather to be with the Dothideaceae. If there were several perithecial cavities in a single stroma it would be very closely related to such plants as *Dothidea ribesia*. The thickened character of the wall at the apex of the ascus which is presented in most species of *Gnomonia* is also present in certain species of *Dothidea* and *Plowrightia*.

The absence of a perithecial wall in the stroma and the origin of the perithecium within the interior of the stroma which first bore the conidia on its surface certainly does not accord with the Sphaeriales. Because of the peculiar character of the fruit bodies of this species and the wide divergence from the structure of members of both the Sphaeriales and Dothideales, it is extremely difficult to determine the exact generic relationships. For the present therefore it may be as well to allow it to remain in the genus in which it was first described.

Since as a result of this study the ascigerous and conidial stages are connected for the first time, the following brief diagnosis is given.

Sphaerella opuntiae E. & E. Jour. Myc. 4: 82. 1888.

Syn. *Gloeosporium lunatum* E. & E. Proc. Acad. Phil. 43: 82. 1891.

Ascigerous stage.

Fruit bodies numerous, black, on both surfaces of the segments, formed in the stomata, wedge shaped, broad above and tapering into the stomatal cavity. Fruit bodies connected laterally by dark brown mycelium. Wall of the fruit body dark brown, thick, 50—100 μ . Perithecial cavity flask-shaped, 100—120 μ in diameter. Asci elongate with thick walls with an apical perforation in the inner wall, 60 $\approx$ 12—15 μ ; spores hyaline, 20—22 $\approx$ 3,5—4 μ , 1-septate, constricted at the septum, and distichate. Appearing on dead areas formed by *Gloeosporium lunatum*.

Conidial stage.

Areas orbicular, 1—3 cm in diameter, isolated, when numerous becoming confluent. Diseased tissue may drop out leaving an opening. Acervuli numerous with a prominent superficial stroma, 60—125 μ in diameter, flesh-colored; conidia lunate-fusoid, 12—20 $\approx$ 2—3 μ , often 1-septate; appearing on *Opuntia* sps. in early spring causing a severe rotting and falling off of segments.

The Black Spot Disease.

The first indication of the presence of this disease is the formation of smoky, circular areas about 5 mm. in diameter. These pale out rather abruptly into the surrounding green tissue leaving the advancing edge of the spot very plainly manifest. These infested areas may be few in number and isolated or more frequently so numerous as to become confluent. At the center of these areas later appear small, black, granular

bodies, the beginnings of the perithecia. The youngest perithecia are thus formed on the youngest part of the mycelium or farthest away from the center of infection. In the advanced stages the carbonaceous perithecia cover the entire area and hence the popular name of „black spot“ is applied to this disease. The margin of the spot at this stage of development becomes bordered by a halo of chlorotic tissue. In severe cases the entire joint is covered by the closely congested perithecia and in other cases isolated areas 1 cm. in diameter become blackened (Fig. 3).



Fig. 3.

The relation of the fungus to the host is shown in a diagrammatic way in Fig. 4 B. The vegetative body of the fungus is entirely within the tissue of the host and the perithecia are formed directly above the stomatal openings. Within the substomatal cavity there is formed a plug of pseudoparenchymatous tissue (Fig. 5 A) which completely fills the opening. The mycelial filaments do not penetrate the epidermal cells, the crystal-bearing hypoderma or the collenchymatous hypoderma, at all, or to only a slight extent. The diseased areas are enlarged by the

ramifications of the mycelium mainly through the palisade parenchyma to the adjacent substomatal chambers. Here the mycelium again fills up the openings and produces its perithecia on the surface of the segment. Upon the same joint may be found infected areas showing all stages in the development of the fungus indicating that new infections may occur on the same segment regardless of their age. Infections for these new areas in all probability takes place through the stomata, the cuticle being very thick and resistant.

The Perithecia.

This fungus belongs to the genus *Perisporium* a member of the Perisporiaceae. The perithecia are the most prominent external evidence of the presence of this fungus. Two species have been described as inhabitants of the *Opuntia*. One, *Perisporium mendozanum* Speg., was originally described¹⁾ from material collected in Argentine where it occurs on

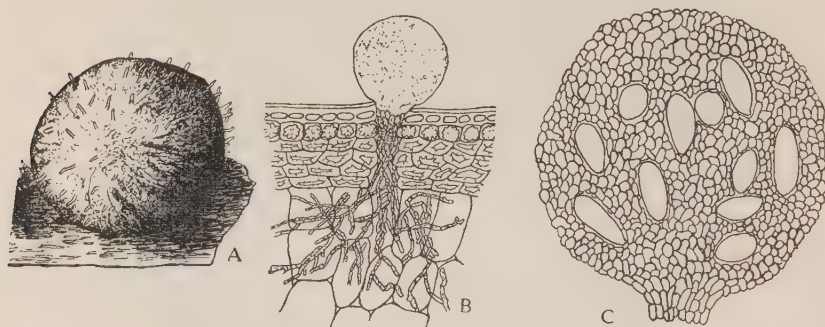


Fig. 4.

the spines of *Opuntia aoracantha*. The other species *Perisporium wrightii* B. & C. was described from material collected in Texas by Charles Wright, one of the pioneer collectors in the State. It lives on the stems or joints of several species of *Opuntia*. The perithecia first appear at the stomatal openings as small spherical masses of pseudoparenchyma. As these increase in size the asci are formed (Fig. 4 C.). They are at first irregularly scattered in the pseudoparenchyma. At maturity a hollow receptacle containing a group of asci is present.

The perithecia are globular in form (Fig. 4 A.), 300–350 μ in diameter and very easily separated from their point of attachment. Short dark setae project from the surface of the perithecialium. The asci are short obovate (Fig. 5 B.), 80–90 μ $\times$ 20–26 μ and the spores 24–30 μ $\times$ 10–14 μ , brown, 3-septate and constricted at the points of septation (Fig. 5 C.). Only rarely could material be found in which the asci and spores were mature. This may account for the fact that the original

¹⁾ Spegazzini, C. An. Soc. Cient. Arg. 227. 1899.

description¹⁾ is very incomplete. The following is the description as given. „Congested, granular, black; asci short obovate; spores (jointed) subglobose, lilac colored contents.“ Immature spores are deep lilac in color becoming brown at maturity.

Numerous attempts were made to germinate the spores in agar plates and in hanging drops of water or agar, but in no case was germination secured.



Fig. 5.

This disease is the least abundant of all the diseases under consideration and in no instance has it been observed to be the cause of any serious injury. It is not generally fatal to the life of the joint and only rarely does the entire plant succumb. New segments adjacent to older diseased ones may show no signs of a disordered condition. While the diseased joint may not be able to perform its normal photosynthetic activities, the vascular system remains intact for a long time so that the segments formed above the infected ones receive their supply of soil water and can function in the normal manner.

¹⁾ Berkeley, M. J. and Curtis, M. A. Cuban Fungi no. 987.

The Sun Scald of Cacti.

Wherever the „Texas pear“ occurs this is the most common and most serious fungous trouble. By the farmers and ranchmen it is commonly called „sun scald“ and its cause attributed to the hot, dry weather. It well merits in external appearance the name by which it is popularly called because of the yellowish brown color which the joints assume. Quite commonly all the joints in the growth of the previous seasons are quite uniformly yellowish brown or grayish brown.

The disease is caused by *Hendersonia opuntiae*¹⁾ E. & E.²⁾. It begins around the areolae of the spines as olivaceous discolorations (Fig. 27). Soon definite spots are formed showing a distinct zonation. The advancing margin is olivaceous and within this is a sharply limited area of isabelline color. The center of the diseased areas are grayish brown and cracked or scaly. These areas are more or less circular at first but as they increase in size the whole segment becomes finally involved. At the time when the grayish coloration has been produced over a part or the whole of the segment, the beginnings of pycnidial formation are evident, appearing under the hand lens as minute, dark specks. These pycnidia mature in the segments only when the latter have become dry and dead. At this time the pycnidia are plainly evident to the unaided eye (Fig. 28). Most commonly the pycnidia are in the same stage of development over the entire segment, although some have been formed on which mature pycnidia occurred in the dry tissue with all the various stages of development in the greener parts of the same segment. The symptomology evidenced by the early stages of this disease is entirely different from the late stages. If a diagnosis were based on a single specimen of the stages shown in Fig. 27 and Fig. 28 they would be pronounced to be caused by different organisms. Plantings of diseased tissue, however, from these stages have been made and the pycnidia of *Hendersonia* developed in these cultures.

Pycnidia.

The fungus apparently gains entrance through the stomata and ramifies through the adjacent epidermal cells. The brown hyphae aggregate in the mouths of the stomata (Fig. 6 A.) and completely close them. It is at this time that the „scalded“ appearance is so characteristic and the stage in which material in the field is most commonly found. These aggregates of hyphae in the stomatal openings are the primordia of pycnidia (Fig. 6 B). As they increase in size the cuticle together with

¹⁾ It is through the kindness of the N. Y. Botanical Gardens that I have been able to examine the type material described by Ellis and Everhart from material collected in Alabama on *Opuntia ficus-indica* and I find that the Texas material on *O. Lindheimeri* is the same.

²⁾ Ellis, J. B. and Everhart, B. M. Jour. Myc. 8: 65. 1902.

the outer wall of the epidermal cells comes to be raised up, permitting the entrance of air and the consequent grayish coloration. By stripping off this outer part, which is now easy to remove from the underlying tissue, a surface view reveals the characteristic growth of the fungus and numerous early stages in the formation of pycnidia. Since part of the vertical walls is broken away, the outline of the epidermal cells appears in surface view. The pycnidia are quite commonly formed underneath the raised cuticle and outer epidermal wall, and above the suberized tissue. When no suberized layer has been formed they may be developed in the elevated cuticle and wholly above the crystal-bearing hypoderma. In other cases as the pycnidium increases in size, the hyphae having extended rather deeply into the stomatal cavity, the crystal-

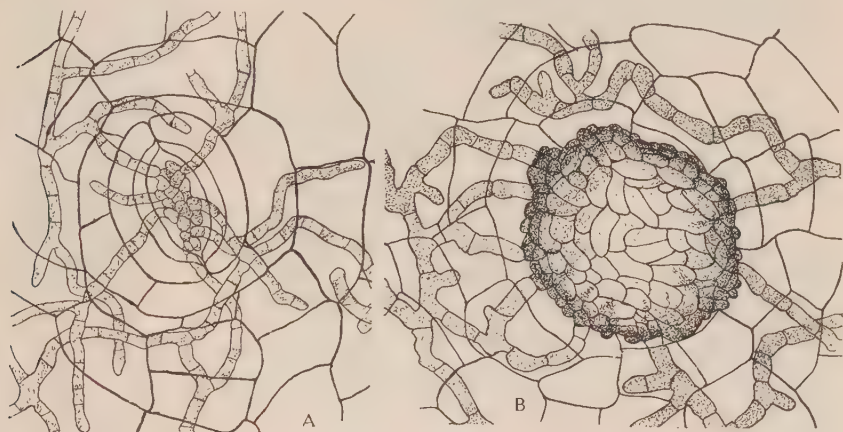


Fig. 6.

bearing cells are pushed upward and apart and the pycnidium comes to be more deeply sunken.

From material which had been soaked in water for several hours, in order to soften it, previous to treating with Gilson's fluid, and which had been stained with eosin, the detail of structure of the pycnidium could be determined. The base and sides of the pycnidial cavity are composed of a thin layer of brown, pseudoparenchymatous cells, about the thickness of the diameter of three cells. At the ostiolum the wall is much more thickened. Short thick conidiophores project from the inner wall and from these the conidia are abstricted (Fig. 7 A). The pycnidia are globular or flattened and 200—300 μ in diameter. The conidia are brown, 3-septate, constricted deeply at the points of septation and $24-30 \approx 4-5 \mu$.

The conidia germinate readily in water or nutrient media (Fig. 7 B). The end cells germinate more frequently than the others, each cell gene-

rally forming only a single germ tube. Occasionally several hyphae arise from a single cell. On glucose agar with the reaction + 10, dense colonies, grayish at the center and whitish or faintly pink at the margin, are developed. These become darker with age and are at length black. At this stage pycnidia are abundantly formed on the surface of the medium. Other media as glycerine agar, lactose agar, and bean pods are just as favorable for the production of pycnidia.

Effect on the Host.

The pathological effect on the host can best be understood by a comparison with the normal morphology. In a healthy mature segment



Fig. 7.

(Fig. 8 A) the epidermis is covered by a thick hard cuticle. Just beneath the epidermis is the first hypodermal layer within nearly all the cells of which is a large calcium oxalate crystal. Beneath this crystal-bearing layer is a region three to five cells deep, the collenchymatous hypodermis¹. The walls of these cells are thick, striate and pitted, appearing to be sclerenchymatous. When treated with concentrated sulfuric acid these cells are soluble. Upon the application of chloroiodide of zinc a violet coloration is produced and with sulfuric acid and iodine a blue color, all of which reactions are due to the presence of cellulose. A cavity extends through the hypodermis to the air chamber in the chlorenchyma.

¹ Ganong, W. F. Present Problems in the Anatomy, Morphology and Biology of the Cactaceae. Bot. Gaz. 20: 129—138. 1895.

The vegetative mycelium is entirely within the epidermal tissue system. The presence of the fungus within the epidermis results in the formation of a suberized tissue. This begins by a division of the epidermal cells parallel to the surface of the segment. The phellogenetic layer gradually moves outward forming at first quite large cells which become more and more compact toward the outside. Very frequently a layer of collenchyma cells is interposed between the cork cells. The stomatal cavities are closed and the pycnidia are raised above these openings (Fig. 8 B). In this way the fungus is kept outside of the fundamental tissue system and a perfect barricade of corky tissue is formed.

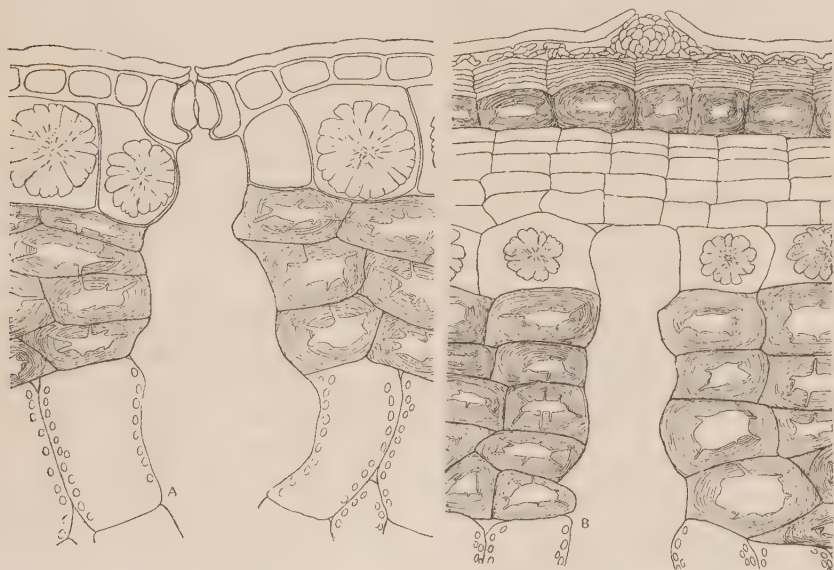


Fig. 8.

In order to see whether the formation of this suberized layer was caused only in response to the presence of the fungus some of the segments were permitted to partially dry out in the laboratory. In these, too, a similar formation of cork resulted. From this it is evident that cork formation is primarily a means of checking water loss. The fungus by lifting up the cuticle and outer epidermal wall, exposes the underlying tissue to desiccation. The plant, then, to protect itself against this loss interposes a suberized layer. The vascular system may function for several years after the segment is infected yet the fundamental system gradually disintegrates. The presence of the plugs of fungus tissue in the stomata and formation of the suberized tissue checks respiration and shuts out the light so that photosynthetic activity is entirely inhibited and the whole segment becomes dry and dead.

Summary.

Three fungous diseases of *Opuntia* are common in Southwestern Texas, *Sphaerella opuntiae* E. & E. whose conidial stage causes an anthracnose, *Perisporium Wrightii* B. & C., the black spot fungus, and *Hendersonia opuntiae* E. & E., the sun scald.

Gloeosporium lunatum is the conidial stage of *Sphaerella opuntiae* E. & E. It produces an anthracnose on *Opuntia* sp. The acervulus is sporodochium-like in appearance and many of the conidia are 1-septate. It should be retained in the genus *Gloeosporium* as an extreme case of the cushion-like acervulus, and since several species of *Gloeosporium* have at least a few septate conidia. Further, it has all the outward appearances of a *Gloeosporium*.

Infection takes place only following the rainy season in the spring, for the most part from new acervuli which are formed on old areas under such conditions. At this time the tissues of the host are but little developed. It causes a serious rotting of the new joints. When it appears at the point of union with the old segment, the newly formed joint falls off.

The collenchymatous hypoderma is extended to check the advance of the fungus. Typically plants form suberized tissue to heal wounds while the *Opuntia* is able to form both suberized and collenchyma tissue for this purpose. Collenchyma is formed also in response to mechanical wounds.

The perfect stage later develops from the stromata of the acervuli. The mass of conidia on the acervulus is pinkish. The stromata become dark colored and the walls thicken after conidia cease to be formed. Mature perithecia appear about a month after the *Gloeosporium* stage has ceased to develop conidia. A thick-walled, dark mass of pseudo-parenchyma cells constitute the fruit body. The perithecial cavity is formed in this stroma. This type of fruit body does not accord with the genus *Sphaerella*.

Perisporium Wrightii B. & C., the black spot fungus, is the least common and causes the least amount of injury to the host. When once the fungus has gained an entrance it spreads through the mesophyll, plugging up the substomatal cavities and forming the perithecia on the surface of the segment. The tissues of the host are not rapidly destroyed, but the segment may remain active for several years. New infections may occur on old segments.

Hendersonia opuntiae E. & E., the sun scald of *Opuntia*, is very abundant. Segments of all ages are affected. The mycelium is within the epidermal tissue system. It closes the stomata and from these stomatal plugs the pycnidia are developed. The „scalded“ appearance is due to the formation of suberized tissue. This develops from a phellogenetic layer formed by a division of the epidermal cells parallel to the surface

of the segment. This suberization is a response of the plant to prevent desiccation and develops independently of the fungus. The disease is very destructive.

Explanation of Textfigures and Plates.

- Fig. 1. Cacti with oaks and mesquite in the background.
- Fig. 2 A. Diagrammatic representation in section of the edge of an anthracnosed area and the adjacent normal tissue $\times 10$.
 a) cuticle, b) collenchymatous hypoderma, c) palisade parenchyma, d) mesophyll, e) mesophyll which has been completely disintegrated, f) stomata from which perithecia are developed, g) partially disintegrated hypoderma, h) a differentiated parenchymatous layer which forms the collenchyma to check the advance of the fungus and heal the wound i) collenchyma tissue, j) the fungus has broken through the first barrier and a second layer of collenchyma has been formed,
- B. Collenchyma tissue from the margin of the wound together with the parenchyma layer from which it is formed $\times 250$.
- Fig. 3. A segment affected with the black spot fungus *Perisporium Wrightii*.
- Fig. 4 A. A perithecium of *Perisporium Wrightii* $\times 75$.
 B. Diagrammatic representation of *Perisporium Wrightii* showing its relation to the host, the mycelium being within the substomatal cavity and chlorenchyma and the perithecium directly above a stoma $\times 50$.
 C. Section through an immature perithecium in which the asci have begun to be differentiated $\times 160$.
- Fig. 5 A. The mycelium of *Perisporium Wrightii* has formed a plug of pseudoparenchyma in the substomatal cavity, the perithecium being broken away $\times 350$.
 B. Asci of *Perisporium Wrightii* $\times 350$.
 C. Several ascospores $\times 350$.
- Fig. 6 A. The radiating mycelium of *Hendersonia opuntiae* beneath the cuticle and outer wall of the epidermal cells. The hyphae have aggregated in a stoma, the beginning of the formation of pycnidia $\times 350$.
 B. The young pycnidium has developed into a spherical mass of pseudoparenchyma $\times 350$.
- Fig. 7 A. Section through a mature pycnidium of *Hendersonia opuntiae*. The pycnidium has been formed above the crystal-bearing hypoderma $\times 350$.
 B. Germinating conidia of *Hendersonia opuntiae* $\times 350$.

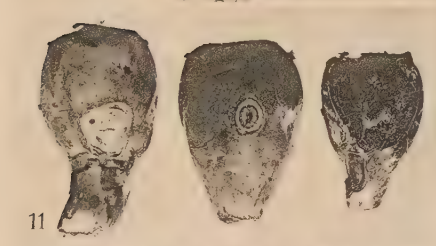
- Fig. 8 A. Section through a normal segment of *Opuntia* $\times 250$.
B. Section through a segment attacked by *Hendersonia opuntiae*. Suberized tissue has been formed with a layer of collenchyma interposed. The pycnidium is being formed above the stomatal chimney $\times 250$.
- Fig. 9. Segment with the characteristic anthracnosed areas caused by *Gloeosporium lunatum* which failed to form acervuli.
- Fig. 10. Perforations formed by the falling out of the anthracnosed areas.
- Fig. 11. Fruit of *Opuntia* attacked by *Gloeosporium lunatum*.
- Fig. 12. An abundant infection of *Gloeosporium* in which only pox-like depressions have been formed over most of the segment.
- Fig. 13. An old segment in which the areas are confluent and the perithecia of *Sphaerella opuntiae* have been abundantly developed.
- Fig. 14. The primary area of an old segment taken from the field has enlarged and new acervuli have been formed.
- Fig. 15. A young acervulus of *Gloeosporium lunatum* which has ruptured the epidermis preparatory to the formation of the superficial stroma $\times 200$.
- Fig. 16. Mature acervulus bearing conidia. The acervuli are for the most part formed in the stomata $\times 200$.
- Fig. 17. Conidia of *Gloeosporium lunatum* taken from an anthracnosed plant $\times 400$.
- Fig. 18. Conidia, taken from an acervulus, germinating in agar $\times 400$.
- Fig. 19. Germinating conidia obtained from pure culture. Conidia are being formed from papilla-like conidiophores $\times 400$.
- Fig. 20. An acervulus which has ceased to form conidia. The cells have become thick-walled and dark colored and the mycelium within the substomatal cavity has become more abundant $\times 200$.
- Fig. 21. A later stage in which the pseudoparenchymatous tissue which is to become the perithecium has completely filled the substomatal chamber $\times 200$.
- Fig. 22. The stroma has pushed the cuticle upward and the hypodermal cells have become badly disintegrated. Certain cells near the center of the stroma have become thin-walled and clear, preparatory to the development of the perithecial cavity $\times 200$.
- Fig. 23. Mature perithecium of *Sphaerella opuntiae* $\times 200$.
- Fig. 24. Asci of *Sphaerella opuntiae* $\times 400$.
- Fig. 25. Ascospores $\times 500$.
- Fig. 26. Germinating ascospores $\times 800$.
- Fig. 27. Segment affected by *Hendersonia opuntiae*. The diseased areas are zonate and pycnidia have begun to be formed.
- Fig. 28. Mature pycnidia of *Hendersonia opuntiae* on a dry segment.
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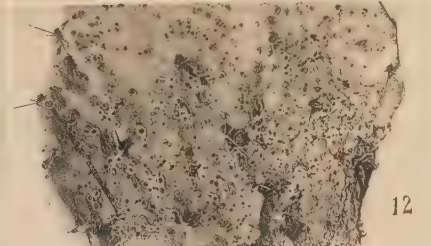
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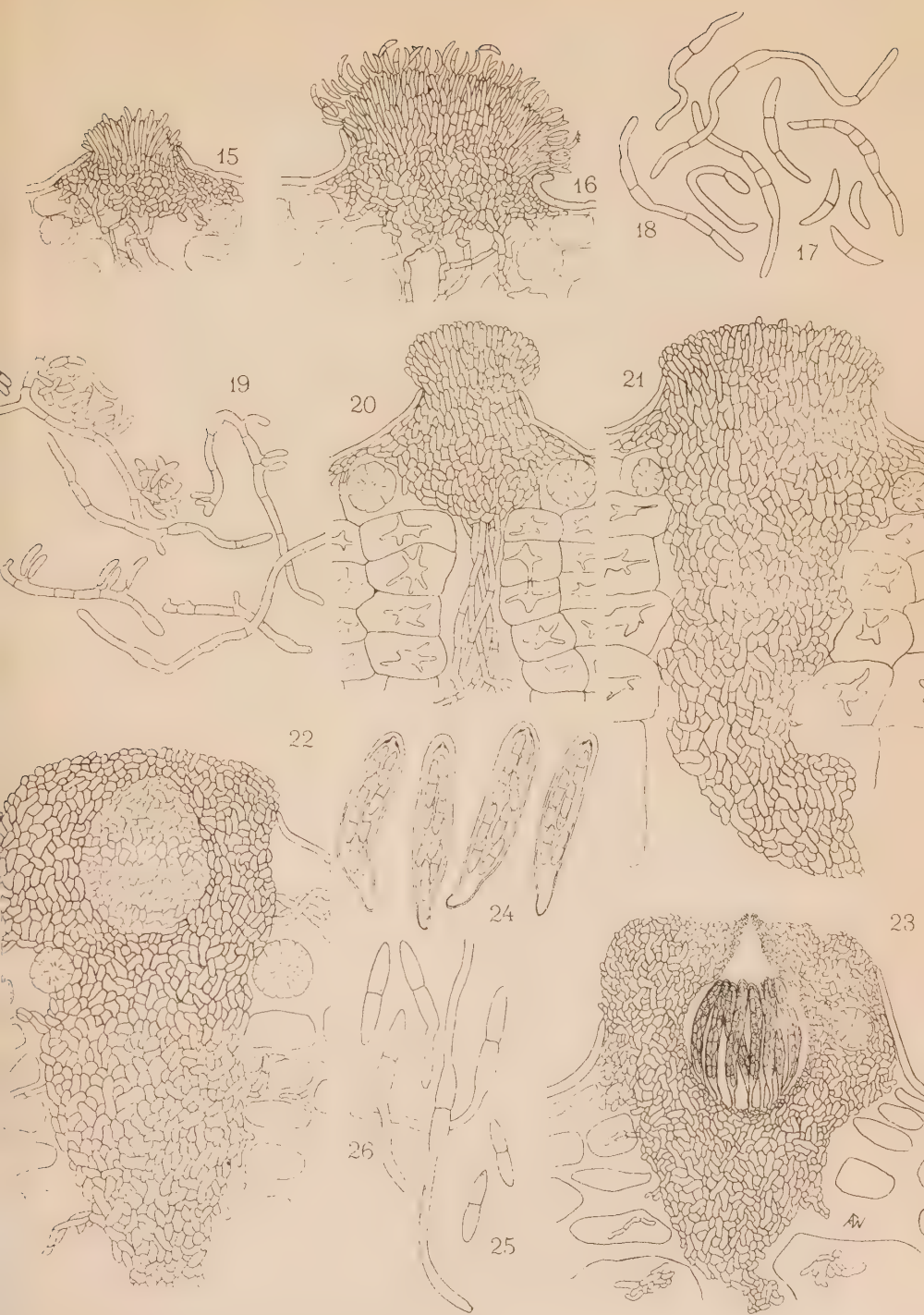
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Reprinted from the "ANNALES MYCOLOGICI"

(vol. IX, No. 6, 1911)

A Disease of the Cultivated Fig, *Ficus Carica* L.¹⁾

By Fred. A. Wolf.

In the month of August, 1910, some partially decayed figs were brought to the botanical laboratory of the University of Texas. The tissue of the fruit was found to contain the mycelium of a fungus, with no evidence, however, of the formation of spores either upon the surface or within the tissue of the fig. Consequently, some of the rotted tissue from the interior of the fruit was transferred with a sterile needle to slant tubes of glucose agar in the hope that the fungus might be induced to develop its fruits. In every case a pure culture of a fungus was obtained, whose mycelium was loose, floccose and whitish at first, becoming grayish and finally inky black. After four days an abundance of pycnidia were developed on the surface of the agar.

At the same time some of the figs had been left intact and others cut open and placed in a sterile moist chamber to permit the further growth of the fungus. Pycnidia were abundantly formed, at first underneath the epidermis, at length breaking through and appearing as minute black specks. On the fruits which had been opened the pycnidia were developed also on the exposed surface. The pycnidia from the fruits as well as from the cultures were found to contain the spores of a species of *Macrophoma*.

Two species of *Macrophoma* have been described as occurring on figs. *Macrophoma sycophila*²⁾ (Massee) Sacc. and D. Sacc. occurs on the dried leaves of *Ficus* sp. in Queensland, Australia. The other one was recently described as a new species, *Macrophoma fici*³⁾ Alm. and Cam., occurring on the dead twigs of *Ficus punctifera* Warb. in Africa. Because the species which I have found on the fruit agrees in morphological characters with this latter species, it is referred to *Macrophoma fici*. No record has been found of its previous occurrence either in America or in any other locality than in Africa. Neither is there any record that this fungus is capable of producing a disease of the fruit.

This disease has been observed only on one of the purple varieties of figs. Because of this color of the fruit the symptoms of the progress

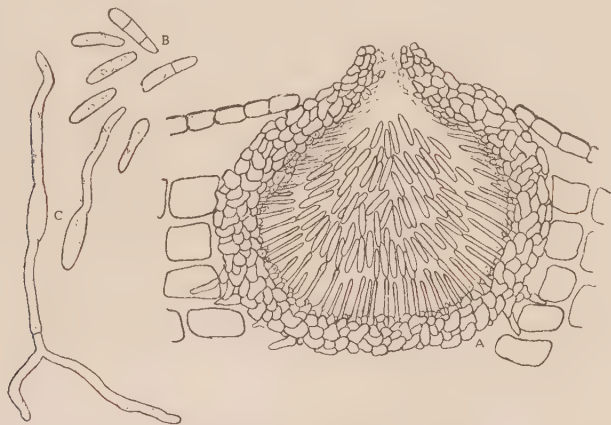
¹⁾ Contribution from the Department of Botany, Cornell University, No. 145.

²⁾ Massee, G., Roy. Bot. Gardens, Kew Bull. 177:156. 1901.

³⁾ d'Almeida, J. V., and d'Souza da Camara, M., *Duas especies mycológicas Africanas*. Revista Agronomica. 4:61. 1906.

of the rot are not prominent. The fungus gains entrance by way of the open end of the receptacle, just at the time that the fruit is beginning to ripen. This end of the fruit becomes slightly yellowish and a small drop of sugary juice is exuded which collects at the opening. Often this discoloration is scarcely noticeable and may be accompanied by a slight depression. The softer nature of the diseased part of the fruit and the presence of the sugary excretion are characters by which the diseased figs can be distinguished from healthy ones. A characteristic odor resembling fermentation accompanies the fungus in the fruit. In the advanced stages the figs are completely decayed and drop from the tree.

The pycnidia vary in size from 150—350 μ . The pycnidial wall consists of dark, thick-walled, parenchymatous cells. The pycnidia are globose or slightly flattened. The spores which are 22—28 $\times$ 10—12 μ



are borne on basidia longer than the spores. These basidia are straight and uniform or slightly clavate.

In order to gain some idea of the manner in which the fungus maintains itself from year to year an examination of the trees bearing diseased fruit was made. It is known that the disease has been present in one grove for several years, resulting in an estimated loss of one third to one half the crop. Since fallen fruit is either eaten by animals or soon completely disintegrates, it was evident that the fungus must harbor in some other substratum. Many of the twigs and tips of the larger branches were found to be dead. Within the dead bark were embedded numerous, black pycnidia whose spores agreed in size with those found on the fruits. In order to determine their identity cultures were made. The resulting mycelial growth was the same as that obtained from the plantings of the diseased tissue of the figs, and within a few days pycnidia appeared in these cultures. The identity was further

established by transferring some of this mycelium to the opening of normal fruits in a moist chamber, care being taken to not injure adjacent tissue. Within three days the entire fruits were invaded by the filaments of the fungus and later pycnidia were formed.

In the original description the range in size of pycnidia is from 250—350 μ . On the twigs they are commonly found to be much smaller being only 150 μ in diameter. Attention may also be called to the fact that the great variation in spores¹⁾ exists also in the spores taken from pycnidia on the decayed fruits. In addition to this wide range of variation in form, occasional spores were observed to be septate sometimes dividing the spore into two unequal halves. In a few rare instances two septa were observed. Since the spores of some fungi become septate before the formation of germ tubes, the presence of these septa might be thought by some to be initial steps in germination. Observations on the germination of *Macrophoma fici*, however, show that the spores germinate in the normal manner. A hypha is formed terminally which soon becomes branched and septate at irregular intervals. The occurrence of two-celled or three-celled spores in *Macrophoma fici* is the exception rather than the rule. For this reason it is felt that no taxonomic significance need be attached to the occasional presence of spores with septa. This variation from the typical unicellular condition need only be considered as one of the peculiar characteristics of this species of *Macrophoma* occurring on the fig.

There occurs on the same twigs a species of *Pleospora* and also of *Phoma*. Both of these have been isolated and grown in pure culture. Neither of them showed, from this culture work, any relation to *Macrophoma fici*.

At present no data is available as to the distribution of the disease nor have any experiments been conducted on methods of control. Judging, however, from the life history of the fungus there is at least one suggestion as to control measures. The fungus is very evidently parasitic on the twigs and larger branches and hibernates within the bark. If these diseased parts were cut off and destroyed early in the season little or no infection need be expected.

Explanation of Figures.

- A. Pycnidium of *Macrophoma fici* occurring within the bark of twigs $\times 225$.
- B. Conidia showing variation in shape and septation $\times 450$.
- C. Germination of conidia $\times 450$.

¹⁾ Sporulis diversiformibus (ovoideis, ellipticis, elliptico-ovoideis, conoideis, vel pyriformibus).

Reprinted from the "ANNALES MYCOLOGICI"

(vol. IX, No. 6, 1911)

A Disease of the Cultivated Fig, *Ficus Carica* L.¹⁾

By Fred. A. Wolf.

In the month of August, 1910, some partially decayed figs were brought to the botanical laboratory of the University of Texas. The tissue of the fruit was found to contain the mycelium of a fungus, with no evidence, however, of the formation of spores either upon the surface or within the tissue of the fig. Consequently, some of the rotted tissue from the interior of the fruit was transferred with a sterile needle to slant tubes of glucose agar in the hope that the fungus might be induced to develop its fruits. In every case a pure culture of a fungus was obtained, whose mycelium was loose, floccose and whitish at first, becoming grayish and finally inky black. After four days an abundance of pycnidia were developed on the surface of the agar.

At the same time some of the figs had been left intact and others cut open and placed in a sterile moist chamber to permit the further growth of the fungus. Pycnidia were abundantly formed, at first underneath the epidermis, at length breaking through and appearing as minute black specks. On the fruits which had been opened the pycnidia were developed also on the exposed surface. The pycnidia from the fruits as well as from the cultures were found to contain the spores of a species of *Macrophoma*.

Two species of *Macrophoma* have been described as occurring on figs. *Macrophoma sycophila*²⁾ (Massee) Sacc. and D. Sacc. occurs on the dried leaves of *Ficus* sp. in Queensland, Australia. The other one was recently described as a new species, *Macrophoma fici*³⁾ Alm. and Cam., occurring on the dead twigs of *Ficus punctifera* Warb. in Africa. Because the species which I have found on the fruit agrees in morphological characters with this latter species, it is referred to *Macrophoma fici*. No record has been found of its previous occurrence either in America or in any other locality than in Africa. Neither is there any record that this fungus is capable of producing a disease of the fruit.

This disease has been observed only on one of the purple varieties of figs. Because of this color of the fruit the symptoms of the progress

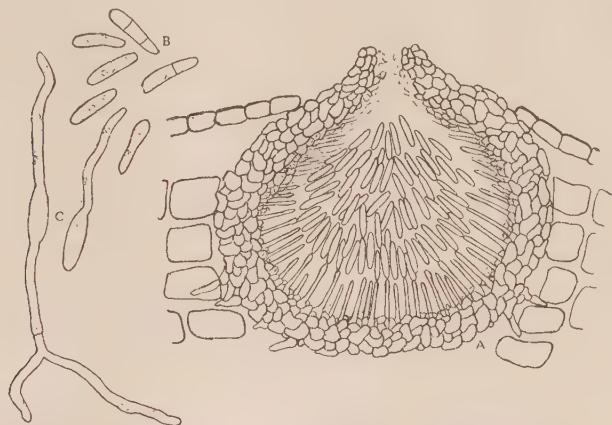
¹⁾ Contribution from the Department of Botany, Cornell University, No. 145.

²⁾ Massee, G., Roy. Bot. Gardens, Kew Bull. 177:156. 1901.

³⁾ d'Almeida, J. V., and d'Souza da Camara, M., Duas especies mycológicas Africanas. Revista Agronomica. 4:61. 1906.

of the rot are not prominent. The fungus gains entrance by way of the open end of the receptacle, just at the time that the fruit is beginning to ripen. This end of the fruit becomes slightly yellowish and a small drop of sugary juice is exuded which collects at the opening. Often this discoloration is scarcely noticeable and may be accompanied by a slight depression. The softer nature of the diseased part of the fruit and the presence of the sugary excretion are characters by which the diseased figs can be distinguished from healthy ones. A characteristic odor resembling fermentation accompanies the fungus in the fruit. In the advanced stages the figs are completely decayed and drop from the tree.

The pycnidia vary in size from 150—350 μ . The pycnidial wall consists of dark, thick-walled, parenchymatous cells. The pycnidia are globose or slightly flattened. The spores which are 22—28 $\times$ 10—12 μ



are borne on basidia longer than the spores. These basidia are straight and uniform or slightly clavate.

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Spore formation in *Podospora anserina* (Rabh.) Winter¹).

By Fred. A. Wolf.

A peculiar interest attaches to the genus *Podospora*²) because of the variation among different species in the number of the functional spores formed, the number ranging from four to sixteen or more in a few species. *Podospora anserina* is one of the tetrasporous species and was made the subject of an investigation for the purpose of observing nuclear phenomena in the formation of the four spores. There has grown up in the last fifteen years a vast literature on nuclear phenomena in the Ascomycetes but the tetrasporous forms have not been particularly investigated.

Podospora anserina is a saprophyte, occurring commonly on the excreta of animals. The fungus which I have studied appeared as a contamination in a poured plate culture of some leaf spot fungi. It is peculiarly adapted

1) Contribution from the Department of Botany, Cornell University, No. 147.

2) The generic name *Podospora* Cesati (in Rabenh. Herb. Mycol. No. 259, and in Hedw. 1, 103, 1852), is employed here for the following reasons. 1st. It has been in use for more than half a century and is employed by Winter in Rabenh. Krypt. Flora, etc., 1, 2d Abth. 169, 1887. 2d. The genus *Pleurage* Fr. (Summa Veg. Scand., 418, 1849), resurrected by O. Kunze (Rev. Gen. Plant. 33, 504, 1898) and used by some recent writers, occupies a very questionable position. Fries merely proposed *Pleurage* in a foot-note, although in the body of the text preceding, on the same page, he uses *Schizothecium* Corda (Icones Fung. 2, 29, pl. 13, 105, 1838). Fries states that he does not know this genus, but that if it is a good one the name ought to be changed on account of *Schizotheca* Ehrh. 1832. According to Art. 57 of the International Rules of Botanical Nomenclature adopted at Vienna in 1905, *Schizothecium* would not be changed on account of an earlier *Schizotheca*. (It may be said in addition that the latter name is regarded as a synonym of another valid genus name in the Alsineae.) Fries' mention of *Schizothecium* Fenzl is a mistake (Professor Farlow informs me that there is no *Schizothecium* Fenzl, and probably Fries meant *Schizothecium* E. Fenzl 1833 in „Versuch einer Darstellung der geographischen Verbreitungs- und Vertheilungsverhältnisse der natürlichen Familie der Alsineen," etc.). *Schizothecium* Corda therefore has precedence over *Pleurage* Fr. It is extremely doubtful if we can now determine what species Corda placed in *Schizothecium*. It therefore seems better, pending the adoption of lists of genera conservanda and excludenda, to employ a genus name which has been in use for many years by specialists in this group.

Geo. F. Atkinson.

to growing on paper but cannot be sectioned successfully on this substratum since the fibers of the paper do not permit the sections to cling in ribbons. If, however, the paper is carefully cut away this difficulty is avoided. When cultured on nutrient agar perithecial formation is readily secured. Small blocks of agar on which the fungus is growing may be cut out, fixed, microtomed and stained. Flemming's medium fixing solution seemed to give the most satisfactory results when used with Haidenhain's iron-alum-haematoxylin or with triple stain. Merkel's fluid and Flemming's strong and weak solutions were also used.

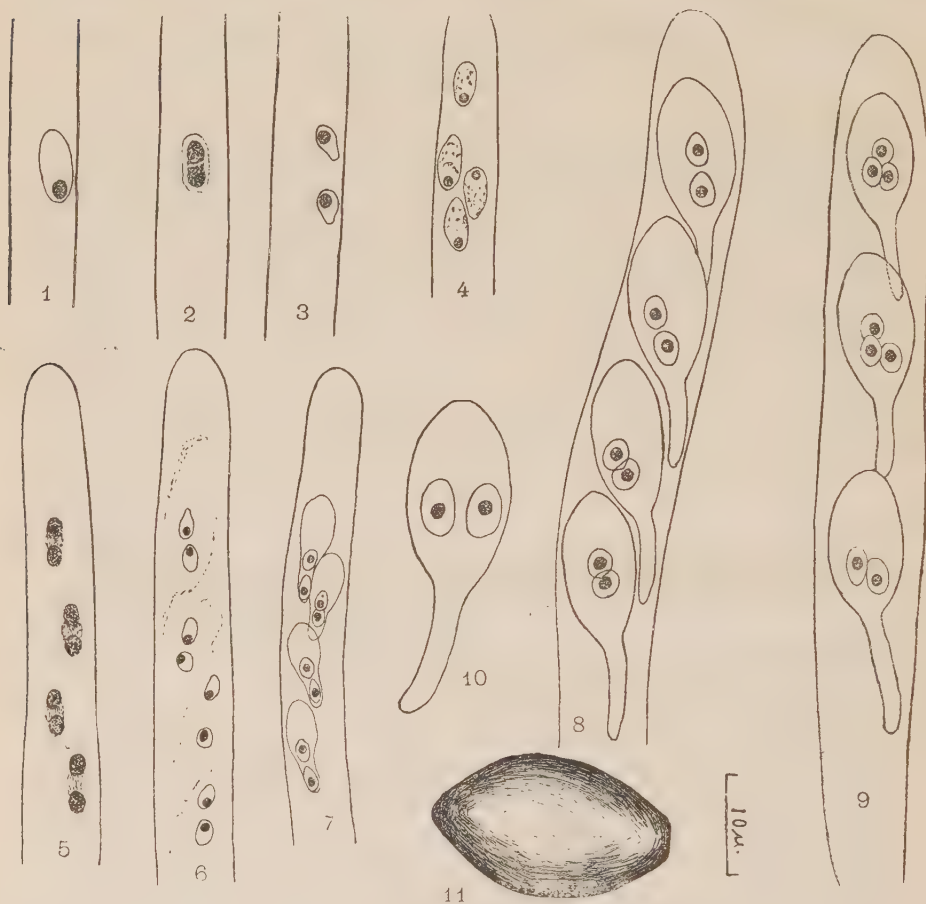
The perithecia arise from hyphal coils. The coiling of the two threads was observed both in living and fixed material. The nuclei are so small, the filaments themselves being only 3μ in diameter, that none of the stages in fusion were observed. That this association of the two filaments represents the sexual process is however very probable. A sphere of parenchymatous tissue develops from this coil. When this structure, the perithecium, has attained about half its mature size the neck begins to be formed, the perithecium being pear shaped at maturity. At this stage of development the asci are formed in the base of the perithecium. The asci arise in a tuft, in young perithecia several being seen to branch from a single hypha. The cells of the perithecial wall, the filiform paraphyses and the vegetative mycelium are multinucleate.

When the ascus has attained about one-third its mature length it contains one large nucleus, the primary nucleus, much larger than those of the vegetative mycelium. Its position is normally near the center of the ascus and its diameter equal to one-half that of the ascus (Fig. 1). That some process of karyokinetic division occurs is quite apparent. But few of the stages could be seen in the preparations used. The first two divisions, probably heterotypic and homeotypic respectively, follow each other in rapid succession. The chromatic material in the binucleated stage is half that of the uninucleated stage and there is but little stainable plasma in the clear court surrounding the nucleolus. Fig. 2 shows one of the final stages in the division of the primary nucleus, the daughter nuclei being nearly formed. Fig. 3 is a later stage in which the nuclei have separated preparatory to the second division. After the second division there is a short period of rest. The nuclei enlarge and knots of chromatin appear but whether these can properly be termed chromosomes is impossible to state (Fig. 4). Brooks¹) finds in *Gnomonia erythrostroma* that the four nuclei rest for some time before undergoing the third division, the chromatin net-work becoming clearly marked. While this rest period of a longer or shorter duration is not specially emphasized by other students of the Ascomycetes yet an examination of their figures

¹) Brooks, F. T., Development of *Gnomonia erythrostroma* Pers. Ann. Bot. 24: 585—605. Pl. XLVIII—XLIX. 1910.

would indicate that it must occur. In *Pyronema confluens*¹⁾ the four nuclei have become much enlarged before the third division, indicating that some time elapses before this last division occurs.

In Fig. 5 is represented one of the late stages in the third division. The chromatin is arranged in two groups some of the nuclei showing



faintly the spindle fibers. The daughter nuclei remain in pairs close together so that at this time there are eight free nuclei within the ascus. Presently hyaline cleavage zones can be seen between the pairs of nuclei (Fig. 6). No clearly defined membrane is laid down but the granular protoplasm is less dense in these zones. The details in the process of

¹⁾ Harper, R. A., Sexual Reproduction in *Pyronema confluens* and the Formation of the Ascocarp. Ann. Bot. 14: 321—400. Pl. XIX—XXI, 1900.

spore delimitation and the subsequent laying down of the spore membrane could not be determined. The fact remains, however, that two nuclei are included within each of the four spores formed, none of the eight free nuclei disintegrating in the epiplasm (Fig. 7—8). In *Phyllactinia*¹⁾ there is a stage in development in which the ascus contains eight free nuclei. As a rule, however, only two spores are formed leaving the six supernumerary nuclei to disintegrate in the epiplasm. One exception to this binucleated condition in *Podospora* was noted. In this case three spores were formed within the ascus (Fig. 9), the two upper ones containing three nuclei each and the lower one two nuclei.

It has been shown also that one-celled ascospores are not regularly uninucleate although Harper²⁾ in his early work believed that they were. The spores of *Tuber aestivum* are multinucleate³⁾. Around four or five of the nuclei and in close proximity to their membranes the cell walls of the ascospores are developed. The nuclei increase in size and number until there are about eight in a spore. Details in the process of spore delimitation for such multinucleate spores are not described. Nuclear division, however, in ascospores without cell division has been pointed out by other investigators⁴⁾.

In *Podospora anserina* whether only one nucleus of each pair takes part in this process of spore delimitation or whether both nuclei are concerned and the astral rays flow together from opposite directions could not be determined. Judging from the fact that the nuclei always lie in the axis of the newly formed spore and near the lower end, it seems quite probable that only the lower one of each pair is concerned in the formation of the wall. The young spores soon become broader at the upper end (Fig. 7). The lower part becomes the hyaline appendage⁵⁾ of the mature spore and the upper part of the dark spore has become prominently enlarged, one nucleus lies within the end which is to become the appendage. As the spores increase in size there is a corresponding growth of the nuclei which come to lie near the center of spore (Fig. 8). When the spores reach the size shown in Fig. 10, they begin to change from hyaline to greenish. This color gradually darkens becoming at length

1) Harper, R. A., Sexual Reproduction and Organization of the Nucleus in Certain Mildews. Carnegie Inst. of Washington Pub. 31: 1—104. Pl. I—VII. 1905.

2) Harper, R. A., Cell Division in Sporangia and Asci. Ann. Bot. 13: 467—525. Pl. XXIV—XXVI. 1899.

3) Dixon, H. H., The Possible Function of the Nucleolus in Heredity. Ann. Bot. 13: 269—278. 1899.

4) Dittrich, C. G., Zur Entwicklungsgeschichte der Helvellineen. Beiträge zur Biol. der Pflanzen. Bd. VIII Heft. 1, 17—52, t. IV—V. 1899.

5) Some spores have an appendage at the apex of the spore in addition to the basal appendage.

dark brown. The appendage which is nearly equal in length to the body of the spore remains hyaline and may entirely disappear in very old spores (Fig. 11).

In a recent paper Lewis¹⁾ has found that eight free nuclei are formed in *Podospora zygospora*. From these the eight uninucleated sporogenous cells are formed. By the development of these cells and the division of the primary spore nucleus, a multinucleated filamentous spore is formed. Septa may or may not be formed. In case they do occur each cell is uninucleate. Each end of the filament may develop into a fertile cell or functional spore. No multinucleated fertile cells were observed. In the case, however, of *Podospora anserina*, the spores are binucleated from the first and no subsequent nuclear division occurs up to the time the mature spore has been formed.

¹⁾ Lewis, I. M., The Development of Spores in *Pleurozia zygospora*. Bot. Gaz. 51: 369—373. Pl. XIX. 1911.

The Brown Leaf spot of Colt's foot, *Tussilago farfara* L.¹⁾.

By Fred. A. Wolf.

The Colt's foot, *Tussilago farfara*, is sometimes subject to a serious leaf spot disease, caused by *Ramularia brunnea* Pk. It appears in the vicinity of Ithaca, New York, in late summer and autumn causing the formation of very characteristic, large, brown spots. The spots are generally orbicular, unequal in size, often confluent and sometimes so numerous as to involve the entire leaf. The conidiophores are formed in fascicles which emerge through the stomata on the upper surface of the leaves. They are so numerous as to give to the leaf an ashy gray color, (Fig. 1). The conidia are cylindrical in form varying from one-to four-celled, (Fig. 2). They are produced in simple or branched chains and range in size from $12-40 \approx 3,5 \mu$. The production of conidia of this type in chains are characteristics of the genus *Septocylindrium* while in *Ramularia* the conidia are not in chains.

This fungus was found to grow readily in bean agar and was isolated in pure culture by the poured plate method. Germ tubes may form equally well from any of the cells of the spore (Fig. 3). There is at first a more or less noticeable increase in size and then the germ tube develops either terminally or laterally. The mycelium soon becomes profusely branched and septate. The development of colonies in bean agar proceeds slowly requiring several weeks to form a colony a few millimeters in diameter, individual colonies never becoming larger than about six millimeters. The mycelium remains closely prostrate forming a densely interwoven grayish white colony. On bean pods hemispherical whitish heaps of mycelium are formed. These later become grayish black. The entire colony can be separated from the pod which has apparently been only slightly changed. Conidial formation is readily secured in these cultures, but no ascospore stage appeared.

Affected leaves were collected at various times during the autumn and put away in wire cages to winter out of doors. In March these were found to contain, embedded in the spots formed by the conidial stage, minute, dark bodies. On April 27th these minute bodies were found to have developed into perithecia. Further study showed that

¹⁾ Contribution from the Department of Botany, Cornell University No. 146.

this perithecial stage agreed morphologically with *Sphaerella tussilaginis* Rehm, which was described as occurring in decaying leaves of *Tussilago farfara*. The perithecia are closely aggregated, either uniformly scattered throughout the spot previously occupied by the conidial stage or in a circle on the border of the spot. The ostium is slightly protruding and opens to the upper surface of the leaf. Large, dark, thick-walled cells made up the perithecial wall. The perithecia vary in diameter from 90—120 μ (Fig. 5). The asci are 40—45 $\times$ 10—12 μ and the spores hyaline, 12—14 $\times$ 3—4 μ and 1-septate (Fig. 6). The spores germinate readily in water (Fig. 7). When the perithecia are crushed the asci escape in a cluster, being united at the base.



On leaves which were placed in a moist chamber tufts of slender conidiophores were developed from the cells of the perithecial wall near the ostium (Fig. 4). These were found to develop conidia like those which had appeared on the spots in the previous summer, showing that *Sphaerella tussilaginis* is the ascigerous stage of *Ramularia brunnea*. Many perithecia were found from which conidiophores and conidia were being formed profusely. When these perithecia were teased apart it was found that the asci and ascospores had not yet developed, thus excluding the possibility of the conidiophores arising from the ascospores. In such preparations their origin from cells of the perithecial wall could easily be seen.

Some authors have reported a similar formation of conidia in the case of the strawberry leaf spot caused by *Sphaerella fragariae* (Tul.)

Sacc. whose conidial stage is *Ramularia Tulasnei* Sacc. Prof. Dudley¹⁾ in studying this organism found that the spores germinate within the ascus so that the conidiophores project from the ostium and that infection in the spring is not due to ascospores, but to the conidia formed in this way. It seems very probable too, that *Sphaerella tussilaginis* does not depend on the dissemination of ascospores for the production of new infections though it is reasonable to expect that infection may in some cases take place by ascospores. An abundant crop of conidia may be produced during two days of moist weather, giving ample opportunity for infection to occur by means of conidia.

Since the ascigerous and conidial stages of this fungus have not been previously connected the following complete description of the species is given.

Sphaerella tussilaginis Rehm Asc. no. 100, Wint. Diagn. p. 10.

Syn. *Ramularia brunnea* Peck. Rept. N. Y. Sta. Museum 30:55. 1877.

Ascigerous stage.

Perithecia membranaceous, epiphyllous, aggregated, immersed, ostium protruding, globose, 90—120 μ in diameter. Asci clavate, 40—45 μ 10—12 μ ; spores elliptical to subfusoid, usually biserial, 2-celled, slightly constricted at the septum, 12—14 μ 3—4 μ . On decaying leaves, in April, on the ground, in the spots formed by the conidial stage the previous year.

Conidial stage.

Spots epiphyllous, large, brown, orbicular, often numerous and confluent, sometimes invading the entire leaf. Hyphae (conidiophores) loosely fasciculate, simple, septate, 4 μ in diameter; fascicles numerous giving an ashy gray color to the spot; conidia hyaline, 12—40 μ 3.5 μ , 1—4-septate, cylindrical, in simple or branched chains. On *Tussilago farfara* appearing also in spring from perithecial wall of immature perithecia.

Fig. 1. Tuft of conidiophores and conidia of *Septocylindrium brunneum* from a leaf in autumn $\times 225$.

Fig. 2. Conidia of *Septocylindrium brunneum* $\times 450$.

Fig. 3. Germination of conidia $\times 450$.

Fig. 4. Section through a perithecium of *Sphaerella tussilaginis* when it was still an undifferentiated mass of pseudoparenchymatous cells, showing the origin of conidiophores from cells near the ostium and chains of conidia $\times 225$.

Fig. 5. Section through a mature perithecium $\times 225$.

Fig. 6. Ascospores of *Sphaerella tussilaginis* $\times 450$.

Fig. 7. Germinating ascospore $\times 450$.

¹⁾ Dudley, W. R. The Strawberry leaf blight, *Sphaerella fragariae* (Tul.) Sacc. Cornell Ag. Exp. Sta. Bull. 14: 1889.

